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(71) Applicants: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 12th floor, 1111 Franklin Street, Oakland, CA 94607–5200 (US). UNIVERSITY OF SOUTHERN CALIFORNIA [US/US]; University Park, Los Angeles, CA 90089 (US).

(72) Inventors: MITCHELL, Malcolm, S.; P.O. Box 676060, Rancho Santa Fe, CA 92067 (US). DEANS, Robert, J.; 415 Furman Drive, Clairemont, CA 91711 (US). MINEV, Boris, R.; 12496 Cavallo Street, San Diego, CA 92130–2734 (US). KAN–MITCHELL, June; P.O. Box 676060, Rancho Santa Fe, CA 92067 (US).

(74) Agents: IMBRA, Richards, J. et al.; Campbell Flores LLP, Suite 700, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).

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(57) Abstract

The present invention provides a substantially purified polypeptide portion of a melanoma associated antigen, MG50 (SEQ ID NO: 2) and substantially purified T cell epitopes of MG50. For example, the invention provides a cytotoxic T cell epitope having the amino acid sequence RPRPEQUEPLP (SEQ ID NO: 4) and a helper T cell epitope having the amino acid sequence CSEQPFPEHTASVQHAD (SEQ. ID NO: 3). The invention also provides antibodies that specifically bind to MG50 or an MG50 T cell epitope and provides antigen binding fragments of such antibodies. Also provided are a substantially purified nucleic acid molecule (SEQ ID NO: 1), which encodes a portion of a melanoma associated antigen, MG50, and nucleic acid molecules encoding MG50 T cell epitopes. Vectors containing such nucleic acid molecules and cells containing such vectors also are provided. For example, antigen presenting cells containing a nucleic acid molecule of the invention are provided. The invention also provides methods of identifying an MG50 melanoma associated antigen in an individual and methods of identifying an immune response against an MG50 melanoma associated antigen in an individual. In addition, the invention provides methods of stimulating T lymphocytes that are reactive against cancer cells expressing an MG50 melanoma associated antigen and provides methods of treating an individual having cancer cells that express an MG50 melanoma associated antigen.

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A MELANOMA ASSOCIATED ANTIGEN, T CELL EPITOPES THEREOF AND METHODS OF USING SAME

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BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to tumor biology and cancer therapy and more specifically to a melanoma associated antigen and T cell epitopes of the antigen, as well as to methods of using such compositions to stimulate an immune response against melanoma cells.

BACKGROUND INFORMATION

The incidence of malignant melanoma has been increasing rapidly. Although ultraviolet radiation is believed to be the primary cause of melanoma, familial occurrence of the disease indicates that hereditary factors also may be involved. Unfortunately, methods for successfully treating melanoma have not kept pace with the increasing incidence.

In general, melanoma, like other cancers, is treated using surgical, chemotherapeutic or, in some cases, radiotherapeutic methods, or combinations of these methods. Surgical methods, however, can be curative only when the melanoma is detected early and has not metastasized. Similarly, radiotherapy, when used, generally only is effective when the tumor is localized. In the majority of cases, however, the melanoma has metastasized by the time it has been diagnosed and,

therefore, chemotherapy is indicated, sometimes in combination with surgery or radiotherapy. However, chemotherapy suffers from the disadvantage that it generally is not specific for the melanoma cells, but also kills rapidly dividing normal cells. In fact, toxicity to normal cells generally limits the dose of chemotherapy that a patient can tolerate. In addition, the cancer cells can become resistant to the chemotherapeutic agent and, therefore, refractory to the treatment. Thus, the duration of response to chemotherapy, radiotherapy or surgery can be too brief.

In theory, immunotherapy holds great promise for treating a cancer such as melanoma, particularly because it can be effective against disseminated disease and because it is expected to be specific only for the cancer cells. Efforts at immunotherapy of melanoma have been attempted using crude vaccines being composed, for example, of "killed" melanoma cells isolated either from the patient to be treated or from another patient, lysates of such cells or cell extracts. For example, a potential therapeutic melanoma vaccine, designated MELACINE, has been formulated from lysates of melanoma cells obtained from two different patients and has produced some positive results when used to treat patients having substantial disease or minimal residual disease. However, use of crude melanoma vaccines in immunotherapy is problematic, for example, because the precise antigenic composition of such vaccines is largely undefined. It is generally believed that more effective immunotherapy requires the identification and isolation of proteins that are expressed relatively specifically by melanoma cells, preferably on their surface, but are not expressed on normal cells. Thus, a need exists to identify melanoma associated antigens. The present invention satisfies this need and provides additional advantages.

SUMMARY OF THE INVENTION

The present invention provides a substantially purified polypeptide portion of a melanoma associated antigen, MG50, comprising the amino acid sequence shown as SEQ ID NO: 2, and provides substantially purified T cell epitopes, comprising a contiguous amino acid sequence of SEQ ID NO: 2, particularly a contiguous sequence within the sequence shown as amino acids 1187 to 1447 of SEQ ID NO: 2. For example, the invention provides cytotoxic T cell epitopes, comprising 8 to 11 contiguous amino acids of SEQ ID NO: 2, such as the cytotoxic T cell epitopes RPRPEQEPLP (SEQ ID NO: 4), DVTSGNTVY (SEQ ID NO: 5) and VLFCAWGTL (SEQ ID NO: 6), and provides helper T cell epitope comprising 12 to 25 contiguous amino acids of SEQ ID NO: 2.

In one embodiment, the invention provides MG50 cytotoxic T cell epitopes fused to a signal peptide or a functional portion thereof, which facilitates presentation of the epitope as a complex with an MHC molecule at the surface of an antigen presenting cell. For example, the invention provides MRYMILGLLALAAVCSARPRPEQEPLP (SEQ ID NO: 21), which is a cytotoxic T cell epitope fused to a signal peptide. In another embodiment, the invention provides a chimeric polypeptide, comprising an MG50 polypeptide encoded by SEQ ID NO: 1 or an MG50 T cell epitope encoded by SEQ ID NO: 1, fused to a second polypeptide, which is not MG50 or an MG50 T cell epitope, wherein the second polypeptide can facilitate detection of the MG50 component, for example, or can render an MG50 T cell epitope immunogenic.

The invention also provides antibodies, or antigen binding fragments thereof, that specifically bind the MG50 melanoma associated antigen (SEQ ID NO: 2) or a

peptide encoded by SEQ ID NO: 1, for example, an MG50 T cell epitope. If desired, an antibody of the invention can specifically bind an MG50 T cell epitope that is fused to a signal peptide or a functional portion thereof or can specifically bind a chimeric polypeptide of the invention. Such antibodies, which can be monoclonal antibodies, are useful, for example, to prepare an anti-idiotypic antibody, which specifically binds to the antibody of the invention and provides a mimic of the MG50 antigen used to raise the antibody.

The invention also provides a substantially purified nucleic acid molecule having the nucleotide sequence shown as SEQ ID NO: 1 (nucleotides 1 to 6848), including subsequences shown as nucleotides 1 to 5509, nucleotides 1 to 3555, nucleotides 1 to 4336, nucleotides 3555 to 4336, nucleotides 3555 to 5509 and nucleotides 3555 to 6848. The invention further provides a substantially purified nucleic acid molecule encoding an MG50 polypeptide comprising SEQ ID NO: 2. In addition, the invention provides nucleic acid molecules encoding MG50 T cell epitopes, such nucleic acid molecules comprising a portion of SEQ ID NO: 1, particularly of nucleotides 1 to 5509 or nucleotides 3555 to 4336 of SEQ ID NO: 1, or comprising a nucleotide sequence encoding a portion of SEQ ID NO: 2, particular amino acids 1187 to 1447 of SEQ ID NO: 2. The invention further provides vectors containing a nucleic acid molecule of the invention, for example, expression vectors or viral vectors, and provides cells containing such vectors. In an embodiment of the invention, antigen presenting cells, which contain and express a nucleic acid molecule of the invention, are provided, such cells which can present an MG50 T cell epitope complexed with an MHC molecule at its surface.

The present invention also provides methods of identifying the presence of an MG50 melanoma associated antigen in an individual, for example, by contacting a biological sample obtained from the individual with an antibody that specifically binds an MG50 antigen, wherein specific binding of the antibody to a component of the sample identifies the presence of the MG50 melanoma associated antigen in the individual. Conversely, the invention provides methods of identifying the presence in an immune response against an MG50 melanoma associated antigen in an individual, by contacting a biological sample obtained from the subject with a peptide encoded by SEQ ID NO: 1 and detecting an immunoeffector function of the sample due to contact with the peptide, thereby identifying the presence of an immune response against an MG50 melanoma associated antigen in the individual. For example, the ability of an MG50 peptide comprising an MG50 T cell epitope to stimulate the proliferation of T cells, which are a component of the biological sample, identifies the presence of an immune response in the individual from whom the sample was obtained.

The invention also provides methods for producing a population of antigen presenting cells that express an MG50 T cell epitope complexed with an MHC molecule on their surfaces. For example, the antigen presenting cells can be contacted with an MG50 melanoma associated antigen comprising the polypeptide encoded by SEQ ID NO: 1 or an MG50 T cell epitope encoded by SEQ ID NO: 1, which can be fused to a signal peptide or a functional portion thereof, if desired. In addition, the antigen presenting cells can be contacted with a nucleic acid molecule encoding an MG50 polypeptide, for example, SEQ ID NO: 1 or a nucleic acid molecule encoding SEQ ID NO: 2, or with a nucleic acid molecule encoding an MG50 T cell epitope, which can be fused to a signal peptide. Accordingly, the invention further provides populations

of antigen presenting cells produced by such a method of the invention and provides methods for stimulating T lymphocytes to react specifically against cancer cells expressing an MG50 melanoma associated antigen by contacting the T cells with such antigen presenting cells.

The invention also provides methods for treating an individual having a cancer in which the cancer cells express an MG50 melanoma associated antigen. For example, such an individual can be treated by administration of antigen presenting cells that express an MG50 T cell epitope complexed with an MHC molecule on their surfaces or can be treated by administration of T lymphocytes that have been stimulated *in vitro* to react with cancer cells expressing an MG50 melanoma associated antigen. In addition, an individual can be treated by administration of a composition comprising an MG50 melanoma associated antigen, or a nucleic acid molecule encoding such an antigen, for example, an MG50 polypeptide encoded by SEQ ID NO: 1 or an MG50 T cell epitope encoded by SEQ ID NO: 1. If desired, the composition can contain an immunostimulatory agent such as an adjuvant, for example, DETOX, or a cytokine, for example, interleukin-2 or interferon-α, or the immunostimulatory agent can be administered separately. The invention also provides methods of preventing the formation of a cancer due to cancer cells expressing an MG50 melanoma associated antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A to 1E show the nucleotide sequence (SEQ ID NO: 1) encoding a portion of the MG50 melanoma associated antigen. The open reading frame is underlined. One potential polyadenylation signal is

double underlined and a second is indicated in bold and underlined.

Figure 2 shows the amino acid sequence (SEQ ID NO: 2) of the MG50 melanoma associated antigen encoded by the open reading frame shown in Figure 1 (SEQ ID NO: 1).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a substantially purified polypeptide portion of a melanoma associated antigen, MG50, comprising the amino acid sequence shown as SEQ ID NO: 2. In addition, the invention provides substantially purified T cell epitopes, comprising a contiguous amino acid sequence of SEQ ID NO: 2 or an amino acid sequence encoded by SEQ ID NO: 1, particularly an amino acid sequence encoded by nucleotides 1 to 5509 of SEQ ID NO: 1, preferably by nucleotides 3555 to 4336 of SEQ ID NO: 1. As used herein, the term "substantially purified," when used in reference to an MG50 polypeptide, means that the polypeptide is relatively free from contaminating lipids, proteins, nucleic acids or other cellular material normally associated with an MG50 polypeptide in a cell. Methods for obtaining a substantially purified MG50 polypeptide of the invention are provided, below.

As disclosed herein, MG50 or a T cell epitope of MG50 is useful for stimulating specific reactivity of immunoeffector cells against cells expressing the MG50 melanoma associated antigen. MG50 is considered a melanoma associated antigen because it is expressed on melanoma cells obtained from different individuals. In addition, MG50 is considered a shared tumor antigen because it is expressed on different types of cancer cells, including, for example, melanoma cells, lung cancer cells and rhabdomyosarcoma cells.

Various melanoma associated antigens, which are shared among melanoma cells in different patients, have been identified. The product of the MAGE-1 gene, the MZ2-E antigen (hereinafter "MAGE-1"), was the first shared melanoma antigen identified (Treversari et al., Immunogenetics 35:145- (1991); van der Bruggen et al., Science 254:1643- (1991)). MAGE-1 was determined to stimulate cytotoxic T cell ("Tc cell") activity and is HLA-A1 restricted (Traversi et al., J. Exp. Med. 176:1453-1457 (1992)). Additional related melanoma antigens subsequently were identified and named MAGE-2, MAGE-3 and MAGE-4 based on their homology to MAGE-1. The MAGE antigens do not appear to be expressed in normal tissues.

MART-1 is another example of a shared human melanoma antigen (Kawakami et al., Proc. Natl. Acad. Sci., *USA* 91:3515-3519 (1994)). MART-1 is expressed in melanoma cells and, to a lesser extent, melanocytes and retina, and, therefore, appears restricted to cells of melanocyte lineage. Unlike the MAGE antigens, which are HLA-A1 restricted, MART-1 is presented in association with HLA-A2 molecules, which are expressed in about 50% of the population. In comparison, only about 10% of the population express HLA-A1 molecules.

MG50 also is a melanoma associated antigen and was cloned from the M1 melanoma cell line by subtractive hybridization; the gene encoding MG50 is present on chromosome 2 (Hutchins et al., Cancer Res. 51:1418-1425 (1991); Weiler, "Molecular Characterization of a Novel Human Melanoma Associated Gene (MG50), Dissertation submitted to the University of Southern California, December, 1993; Weiler et al., Genomics 22:243-244 (1994); Genome Data Bank, Accession No.: locus D2S448 (G00-252-144), each of which is incorporated herein by reference). As disclosed herein, the MG50 gene, which

encodes the polypeptide shown as SEQ ID NO: 2, contains cryptic coding sequences located downstream of a polyadenylation signal.

A nucleic acid molecule encoding MG50 was cloned and, early in the sequencing of the cDNA, a peptide, CSEQPFPEHTASVQHAD (SEQ ID NO: 3) was prepared based on the MG50 cDNA sequence (see Weiler, *supra*, 1993; referred to a "pep-50"). This peptide, which was suspected of being encoded by a nucleic acid sequence located downstream of the MG50 coding sequence, stimulated proliferation of melanoma specific T cells (Weiler, *supra*, 1993). As disclosed herein, CSEQPFPEHTASVQHAD (SEQ ID NO: 3) is encoded by a cryptic MG50 coding sequence, as is the peptide RPRPEQEPLP (SEQ ID NO: 4), which also stimulates proliferation of melanoma specific T cells. Remarkably, RPRPEQEPLP (SEQ ID NO: 4) and CSEQPFPEHTASVQHAD (SEQ ID NO: 3) are not encoded by the same reading frame of SEQ ID NO: 1. Specifically, while RPRPEQEPLP (SEQ ID NO: 4) is encoded by the same reading frame as the remainder of the MG50 polypeptide, although it is downstream of a potential polyadenylation signal, CSEQPFPEHTASVQHAD (SEQ ID NO: 3) is out of frame with respect to the MG50 coding sequence. Thus, T cell epitopes of MG50 can be identified in cryptic coding regions of SEQ ID NO: 1 and can comprise an amino acid sequence encoding by various reading frames of the cryptic coding sequence.

RPRPEQEPLP (SEQ ID NO: 4) is a T cell epitope that is recognized by cytotoxic T cells in the context of the Class I MHC molecule HLA-B7. Additional MG50 peptide sequences having the characteristics of T cell epitopes that are recognized by HLA-A1 MHC molecules (SEQ ID NO: 5) or by HLA-A2 MHC molecules (SEQ ID NOS: 6-17) also have been identified (see Table 1). Such MG50 epitopes were identified by homology to consensus HLA-A1 or HLA-A2

epitopes (see Kaat et al., J. Immunol. 152:3904-3912 (1994); Falk and Rotzschko, Sem. Immunol. 5:81-94 (1993), each of which is incorporated herein by reference). The availability of MG50 T cell epitopes that are presented in the context of HLA-A2 MHC molecules provides that advantage that HLA-A2 molecules are expressed by a relatively large number of individuals as compared to HLA-B7.

The invention provides cytotoxic T cell epitopes, comprising 8 to 11 contiguous amino acids encoded by SEQ ID NO: 1, for example, the cytotoxic T cell epitopes RPRPEQEPLP (SEQ ID NO: 4), DVTSGNTVY (SEQ ID NO: 5) and VLFCAWGTL (SEQ ID NO: 6). As used herein, the term "cytotoxic T cell epitope" means a T cell epitope that is recognized by and stimulates cytotoxic T cells. Also provided are helper T cell epitopes, comprising 12 to 25 contiguous amino acids encoded by SEQ ID NO: 1. As used herein, the term "helper T cell epitope" means a T cell epitope that is recognized by and stimulates helper T cells.

As used herein, the term "T cell epitope" means a peptide that is complexed with an MHC molecule and, when complexed with the MHC molecule, can be bound to a T cell receptor. In addition, the term "T cell epitopic fragment" is used herein to mean a peptide portion of a protein, which can be formed due to proteolysis of the protein, that has the characteristics of a T cell epitope as defined above. In view of these definitions, it should be recognized that the terms "T cell epitope" and "T cell epitopic fragment" often can be used synonymously. However, while a "T cell epitopic fragment" specifically comprises a peptide sequence that is present in a protein, a "T cell epitope" can comprise a peptide containing an amino acid sequence that is the same as or different from the corresponding sequence

present in the protein from which the epitope was derived. Thus, the term "T cell epitope" broadly encompasses a T cell epitopic fragment. In addition, the term "peptide" or "peptide portion," when used in reference to MG50, means an amino acid sequence of at least two contiguous amino acids of SEQ ID NO: 2 (amino acids 1 to 1497), particularly of amino acids 1187 to 1447 of SEQ ID NO: 2, and that are unique to MG50.

A T cell epitope varies in size based on the MHC molecule that binds the epitope. Specifically, class I MHC molecules bind peptides containing about 8 to 11 amino acids, generally peptides containing 8 to 10 amino acids and, most often, 9 or 10 amino acids. In comparison, class II MHC molecules bind peptides containing about 12 to 25 amino acids, generally peptides containing 13 to 18 amino acids.

Class I MHC molecules are, or can be, expressed by all nucleated cells, including antigen presenting cells (see below), and present T cell epitopes to Tc cells. The epitopes presented by class I MHC molecules often are produced by proteolysis of endogenously expressed proteins, including proteins expressed in virally infected cells and in tumor cells. The epitope likely associates with the class I molecule in the endoplasmic reticulum, then the complex is transported to the cell surface. Tc cells, which express the CD8 surface antigen ("$CD8^+$") and a T cell receptor, then bind the epitope associated with the class I molecule, thereby activating the effector function of the Tc cells (see, generally, Kuby, "Immunology" 3d ed. (W.H. Freeman and Co., 1997)).

In comparison to class I molecules, which are expressed on by nucleated cells, class II MHC molecules only are expressed by antigen presenting cells (APC's),

including B lymphocytes ("B cells"), dendritic cells, mononuclear phagocytic cells, macrophages, including Langerhans cells and, in humans, venular endothelial cells, and present a T cell epitope to helper T cells ("Th cells"), stimulating the Th cells, such stimulation being effective in immunity to tumors (see, generally, Abbas et al., "Cellular and Molecular Immunology," 2d ed. (W.B. Saunders Co. 1995); Jones and Mitchell, Trends Biotechnol. 14:349-355 (1996), each of which is incorporated herein by reference; see, also, Kuby, *supra*, 1997). Thus, APC's express both class I and class II MHC molecules and, therefore, can activate Tc cells and Th cells.

The epitopes that are bound by class II molecules generally are derived by proteolysis of exogenous proteins, which are internalized in the APC by phagocytosis or endocytosis. In addition, APC's, such as macrophages, can express co-stimulatory B7 molecules, B7-1 (CD80) and B7-2 (CD86), which are recognized by a cell surface molecule (CD28) that is expressed by certain T cells, including naive T cells, and is involved in activation of the T cells. Binding of a T cell epitope and B7 molecule by Th cells stimulates activation of two subsets of Th cells, Th1 cells, which express interleukin-2 (IL-2), interferon-γ, tumor necrosis factor-β and tumor necrosis factor-α and are involved in the cell-mediated immune functions, including activation of Tc cells; and Th2 cells, which secrete IL-4, IL-5, IL-6 and IL-10 and are involved in the activation of B cells (Quan and Mitchell, in "Current Research and Clinical Management of Melanoma" (Kluwer Academic Publ. 1993), which is incorporated herein by reference; see pages 257-277; see, also, Kuby, *supra*, 1997; chaps. 1, 10 and 12; Jones and Mitchell, *supra*, 1996).

In an embodiment of the invention, MG50 cytotoxic T cell epitopes fused to a signal peptide or a functional portion thereof are provided. Fusion of a signal peptide, for example, to the N-terminus of a cytotoxic MG50 T cell epitope, can facilitate presentation of the epitope as a complex with an MHC molecule at the surface of an antigen presenting cell (see Minev et al., Cancer Res. 54:4155-4161 (1994), which is incorporated herein by reference). For example, the invention provides MRYMILGLLALAAVCSARPRPEQEPLP (SEQ ID NO: 21) and MTNKCLLQIALLLCFSTTALSRPRPEQEPLP (SEQ ID NO: 22), which contain the cytotoxic T cell epitope, RPRPEQEPLP (SEQ ID NO: 4), fused to two different signal peptides (signal peptide is underlined).

Signal peptides are well known in the art and consist generally of three functional portions: a basic N-terminal region of about 1 to 3 positively charged amino acids; a central hydrophobic region of about 8 to 12 hydrophobic amino acids; and a polar C-terminal region of about 5 to 7 amino acids with higher average polarity than the central hydrophobic region. Recognition of a signal peptide by a signal peptidase, which is located within the endoplasmic reticulum of a eukaryotic cell, results in cleavage of the signal peptide from the remainder of the molecule, for example, the MG50 T cell epitope.

For use in the present invention, a signal peptide or a functional portion thereof can be based on any naturally occurring signal sequence or can be a non-naturally occurring sequence having the general characteristics of a signal peptide or of a functional portion of the signal peptide. As used herein, the term "functional portion," when used in reference to a signal peptide, means the basic N-terminal region of about 1 to 3 positively charged amino acids; the central hydrophobic

region of about 8 to 12 hydrophobic amino acids; or polar C-terminal region of about 5 to 7 amino acids with higher average polarity than the central hydrophobic region. As disclosed herein, the substitution, for example, of the central hydrophobic 8 to 12 amino acids of a signal peptide with an MG50 T cell epitope having the appropriate hydrophobicity, provides a unique type of T cell epitope having characteristics of an MG50 T cell epitope fused to a signal sequence. Contact of an APC with such an MG50 T cell epitope results in efficient transport of the epitope complexed with an MHC molecule to the surface of an APC.

In another embodiment, the invention provides a chimeric MG50 polypeptide, comprising an MG50 polypeptide, comprising SEQ ID NO: 2, or a peptide portion of MG50 such as an MG50 T cell epitope encoded by SEQ ID NO: 1, fused to a second peptide or polypeptide, which is not MG50 or a peptide portion of MG50. A chimeric MG50 polypeptide provides certain advantages. For example, where the second polypeptide is an enzyme, such as alkaline phosphatase, horseradish peroxidase or luciferase, detection of the MG50 component of the fusion is facilitated by detecting the presence of the enzyme activity. Other such detectable markers, for example, a FLAG epitope, also can be fused to an MG50 T cell epitope or, if desired, to an MG50 polypeptide, for the purpose of detecting the presence of the MG50 or MG50 T cell epitope. Such a detectably labeled chimeric polypeptide is useful, for example, in an immunoassay to identify the presence of anti-MG50 antibodies or of MG50 reactive immunoeffector cells in a biological sample obtained from a subject.

A chimeric polypeptide of the invention also can be MG50 or an MG50 T cell epitope fused to a second polypeptide such as glutathione-S-transferase (GST) or

the His-6 peptide. Such a chimeric polypeptide can be particularly useful for purifying the MG50 or MG50 T cell epitope. For example, GST readily binds to glutathione, which can be attached to an insoluble matrix, thereby providing a simple affinity chromatography method of purifying a GST-MG50 chimeric polypeptide. Similarly, the His-6 sequence readily binds to a cation such as nickel ion, thus allowing for purification of a His-6-MG50 chimeric polypeptide. Such chimeric polypeptides also can be used to purify antibodies that specifically bind to an MG50 component of the chimeric polypeptide.

A chimeric polypeptide of the invention also can be a peptide portion of the MG50 polypeptide, for example, an MG50 T cell epitope, fused to a carrier protein such as bovine serum albumin, bovine gamma-globulin, human gamma-globulin, keyhole limpet hemocyanin, or ovalbumin. Such a chimeric polypeptide can render a haptenic MG50 peptide immunogenic and, therefore, can be useful for inducing an anti-MG50 antibody response, thus providing a means for obtaining anti-MG50 antibodies (see Harlow and Lane, "Antibodies: A laboratory manual" (Cold Spring Harbor Laboratory Press 1988), which is incorporated herein by reference; see, also, Kuby, *supra*, 1997).

MG50, an MG50 T cell epitope, an MG50 T cell epitope fused to a signal sequence, or a chimeric MG50 polypeptide can be produced by a variety of routine methods, including, as appropriate, biochemical purification, recombinant DNA methods or chemical synthesis. An MG50 polypeptide or MG50 T cell epitope, for example, can be produced by recombinant DNA methods (see, generally, Sambrook et al., Sambrook et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1989), which is incorporated

herein by reference). For example, an MG50 T cell epitope can be produced by cloning a nucleic acid encoding the epitope into an expression vector such as a baculovirus vector, then isolating the expressed epitope from an appropriate insect host cell. In addition, an MG50 polypeptide also can be expressed in a mammalian cell, where it can be post-translationally modified in a manner expected for a native MG50 protein. Appropriate expression vectors and host cells are well known in the art (see Kriegler, Gene Transfer and Expression, A Laboratory Manual, W.H. Freeman and Co., 1991, which is incorporated herein by reference) and are commercially available.

An MG50 T cell epitope, for example, can be synthesized using well known chemical methods, including, for example, automated solid phase methods. Chemical synthesis of an MG50 T cell epitope can be particularly desirable because the method allows for the introduction of amino acid analogs such a (D)-amino acids into the peptide, if desired. The incorporation of a (D)-amino acid, for example, can increase the stability of the T cell epitope, which can be particularly useful for preparing a vaccine or for preparing a diagnostic kit.

Native MG50 protein can be purified from a melanoma cell lysate using, for example, an antibody of the invention. In addition, MG50 T cell epitopes can be purified from antigen presenting cells obtained from an individual suffering from a cancer that expresses MG50. Methods for obtaining such T cell epitopes are well known in the art and include immunoaffinity chromatography, gel filtration chromatography or gel electrophoresis (see, for example, Hunt et al., Science 256:1817-1820 (1992); Chicz et al., Nature 358:764-768 (1992); see, also, Deutscher, Guide to Protein Purification (Academic Press,

Inc. 1990), each of which is incorporated herein by reference.

The invention also provides antibodies, or antigen binding fragments thereof, that specifically bind to an MG50 melanoma associated antigen comprising SEQ ID NO: 2, particularly to amino acids 1187 to 1447 of SEQ ID NO: 2, or to a peptide encoded by SEQ ID NO: 1, for example, an MG50 T cell epitope. If desired, an antibody of the invention can specifically bind an MG50 T cell epitope that is fused to a signal peptide or a functional portion thereof or can specifically bind a chimeric polypeptide of the invention. Such antibodies, which can be monoclonal antibodies, are useful, for example, to prepare an anti-idiotypic antibody, which specifically binds to the antibody of the invention and provides a mimic of the MG50 antigen used to raise the antibody. The antibody also can be labeled with a detectable label, for example, a radionuclide, biotin, or an enzyme, using known methods (see, Harlow and Lane, *supra*, 1988; Hermanson, "Bioconjugate Techniques" (Academic Press 1996), which is incorporated herein by reference).

As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. An antibody of the invention, or an antigen binding fragment thereof, is characterized in that it specifically can bind with an MG50 epitope with an affinity of at least about 1×10^5 M^{-1} and, generally, at least about 1×10^6 M^{-1}. Such antigen binding fragments of an antibody include, for example, Fab, $F(ab')_2$, Fd and Fv fragments that retain specific binding activity for an MG50 epitope.

An antibody of the invention can be a either naturally occurring or non-naturally occurring, and can

include, for example, a single chain antibody, a chimeric, bifunctional and humanized antibody, as well as an antigen-binding fragment thereof. A non-naturally occurring antibody can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains (see Huse et al., Science 246:1275-1281 (1989), which is incorporated herein by reference). These and other methods of making, for example, a chimeric, humanized, CDR-grafted, single chain, or bifunctional antibody are well known to those skilled in the art (Hoogenboom et al., U.S. Patent No. 5,564,332, issued October 15, 1996; Winter and Harris, Immunol. Today 14:243-246 (1993); Ward et al., Nature 341:544-546 (1989); Harlow and Lane, *supra*, 1988); Hilyard et al., Protein Engineering: A practical approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995); each of which is incorporated herein by reference).

Methods for raising polyclonal antibodies, for example, in a rabbit, goat, mouse or other mammal, are well known in the art. In addition, monoclonal antibodies can be obtained using methods that are well known and routine in the art (Harlow and Lane, *supra*, 1988). Essentially, spleen cells from a mouse immunized with MG50 or a peptide portion thereof can be fused to an appropriate myeloma cell line such as SP/02 myeloma cells to produce hybridoma cells. Cloned hybridoma cell lines can be screened using labeled antigen to identify clones that secrete the desired monoclonal antibodies. Hybridomas expressing, for example, anti-MG50 monoclonal antibodies having a desirable specificity and affinity can be isolated and utilized as a continuous source of the antibodies, which are useful, for example, for preparing standardized kits containing the antibody.

Similarly, a recombinant phage that expresses, for example, a single chain anti-MG50 antibody provides a monoclonal antibody that can used for preparing a kit.

A monoclonal antibody specific for MG50 or a peptide portion of MG50 can be used to prepare anti-idiotypic antibodies, which present an epitope that mimics the epitope recognized by the monoclonal antibody used to prepare the anti-idiotypic antibodies. Where the epitope to which the monoclonal antibody includes, for example, an MG50 T cell epitope, the anti-idiotypic antibody can be useful for detecting the presence of MG50 reactive T cells in a biological sample obtained from an individual. In addition, vaccines containing anti-idiotypic antibodies can have antitumor prophylactic effects and induce the involvement of Th cells (Mitchell, Brit. Med. Bull. 51:631-646 (1995); Quan et al., J. Clin. Oncol. 15:2103-2110 (1997), each of which is incorporated herein by reference).

The invention also provides a substantially purified nucleic acid molecule having the nucleotide sequence shown as SEQ ID NO: 1 (see, also, Weiler, *supra*, 1993). In particular, the invention provides subsequences of SEQ ID NO: 1, including nucleotides 1 to 5509, nucleotides 1 to 3555, nucleotides 1 to 4336, nucleotides 3555 to 4336, nucleotides 3555 to 5509 and nucleotides 3555 to 6848. The portion of SEQ ID NO: 1 shown as nucleotides 1 to 5510 also is available at GenBank Accession No. D86983 (submitted by N. Nomura; August 2, 1996). As used herein, the term "substantially purified," when used in reference to a nucleic acid molecule of the invention, means that the nucleic acid molecule is relatively free from contaminating lipids, proteins, nucleic acids or other cellular material normally associated with a nucleic acid molecule in a cell. A substantially purified nucleic acid molecule of

the invention can be obtained by chemical synthesis of the nucleotide sequence shown as SEQ ID NO: 1 or by cloning the molecule using, for example, a method of the polymerase chain reaction (PCR), wherein appropriate primers are selected based on SEQ ID NO: 1.

Due to the degeneracy of the genetic code and in view of the disclosed MG50 amino acid sequence shown in SEQ ID NO: 2, particularly of amino acids 1187 to 1447 of SEQ ID NO: 2, additional nucleic acid molecules of the invention would be well known to those skilled in the art. Such nucleic acid molecules have a nucleotide sequence that is different from the sequence shown as nucleotides 1 to 4488, particularly of nucleotides 3555 to 4336, of SEQ ID NO: 1 but, nevertheless, encode the amino acid sequence shown as amino acids 1 to 1497, particularly amino acids 1187 to 1447, respectively, of SEQ ID NO: 2. Thus, the invention provides a nucleic acid molecule comprising a nucleotide sequence encoding an MG50 polypeptide comprising SEQ ID NO: 2. In addition, the invention provides nucleic acid molecules encoding MG50 T cell epitopes, such nucleic acid molecules comprising a portion of SEQ ID NO: 1 or comprising a nucleotide sequence encoding a portion of SEQ ID NO: 2.

As used herein, the term "a nucleic acid molecule encoding," when used in reference to MG50 or to a peptide portion of MG50, including an MG50 T cell epitope, indicates 1) the polynucleotide sequence of one strand of a double stranded DNA molecule comprising the nucleotide sequence that codes for MG50 or a peptide portion of MG50 and can be transcribed into an RNA that encodes MG50 or the peptide, or 2) an RNA molecule, which can be translated into MG50 or a peptide portion thereof. It is recognized that a double stranded DNA molecule also comprises a second polynucleotide strand that is

complementary to the coding strand and that the disclosure of a polynucleotide sequence comprising a coding sequence necessarily discloses the complementary polynucleotide sequence. Accordingly, the invention provides polynucleotide sequences, including, for example, polydeoxyribonucleotide or polyribonucleotide sequences that are complementary to the nucleotide sequence shown as SEQ ID NO: 1 or to a nucleic acid molecule encoding MG50, comprising the amino acid sequence shown as SEQ ID NO: 2, or to a peptide portion of SEQ ID NO: 2. As used herein, the term "polynucleotide" is used in its broadest sense to mean two or more nucleotides or nucleotide analogs linked by a covalent bond.

The invention also provides nucleotide sequences of SEQ ID NO: 1, particularly of nucleotides 3555 to 4336 of SEQ ID NO: 1, which specifically hybridize to a nucleic acid molecule encoding MG50. It is recognized, for example, that SEQ ID NO: 1 shares regions of homology with nucleic acid molecules encoding mammalian peroxidases and Drosophila peroxidasin. Thus, a nucleotide sequence of SEQ ID NO: 1, which is considered to be within the claimed invention, hybridizes under stringent hybridization conditions to a nucleotide sequence encoding MG50, but not to a nucleic acid molecule encoding a mammalian peroxidase, such as those disclosed as GenBank Accession Numbers X15313, X15378, M29907, X14346, L77979, or the like, or to a nucleotide sequence encoding Drosophila peroxidasin, such as that disclosed as GenBank Accession No. U11052.

A nucleotide sequence of the invention is useful, for example, as a probe, which can hybridize to a nucleic acid molecule encoding MG50 and allow the identification of the nucleic acid molecule in a sample. A nucleotide sequence of the invention is characterized,

in part, in that it is at least nine nucleotides in length, such sequences being particularly useful as primers for PCR, and can be at least fourteen nucleotides in length or, if desired, at least seventeen nucleotides in length, such nucleotide sequences being particularly useful as hybridization probes, although such sequences also can be used for PCR. In addition, a nucleotide sequence of the invention comprises at least six nucleotides, preferably at least nine nucleotides, 5' to nucleotide 5509 of SEQ ID NO: 1, where SEQ ID NO: 1 is shown in the conventional manner from the 5'-terminus (Figure 1A; upper left) to the 3'-terminus, most preferably at least nine contained within nucleotides 3555 to 4336 of SEQ ID NO: 1.

The invention also provides vectors comprising a nucleic acid molecule of the invention and host cells, which are appropriate for maintaining such vectors. Vectors, which can be cloning vectors or expression vectors, are well known in the art and commercially available. An expression vector comprising a nucleic acid molecule of the invention, which can encode, for example, MG50 or a T cell epitope thereof, can be used to express the nucleic acid molecule in a cell.

In general, an expression vector contains the elements necessary to achieve, for example, sustained transcription of the nucleic acid molecule, although such elements also can be inherent to the nucleic acid molecule cloned into the vector. In particular, an expression vector contains or encodes a promoter sequence, which can provide constitutive or, if desired, inducible expression of a cloned nucleic acid sequence, a poly-A recognition sequence, and a ribosome recognition site, and can contain other regulatory elements such as an enhancer, which can be tissue specific. The vector also contains elements required for replication in a

procaryotic or eukaryotic host system or both, as desired. Such vectors, which include plasmid vectors and viral vectors such as bacteriophage, baculovirus, retrovirus, lentivirus, adenovirus, vaccinia virus, semliki forest virus and adeno-associated virus vectors, are well known and can be purchased from a commercial source (Promega, Madison WI; Stratagene, La Jolla CA; GIBCO/BRL, Gaithersburg MD) or can be constructed by one skilled in the art (see, for example, Meth. Enzymol., Vol. 185, D.V. Goeddel, ed. (Academic Press, Inc., 1990); Jolly, Canc. Gene Ther. 1:51-64 (1994); Flotte, J. Bioenerg. Biomemb. 25:37-42 (1993); Kirshenbaum et al., J. Clin. Invest 92:381-387 (1993), which is incorporated herein by reference).

A nucleic acid molecule, either alone or contained a vector, can be introduced into a cell by any of a variety of methods known in the art (see Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1994); Chang, Somatic Gene Therapy, Chap. 11 (CRC Press, Inc., 1995), each of which is incorporated herein by reference; see, also, Sambrook et al., *supra*, 1989). Such methods include, for example, transfection, lipofection, microinjection, electroporation and infection with recombinant viral vectors or the use of liposomes. Introduction of a nucleic acid molecule by infection with a viral vector is particularly advantageous in that it can efficiently introduce the nucleic acid molecule into a cell *ex vivo* or *in vivo* (see, for example, U.S. Patent No. 5,399,346, issued March 21, 1995, which is incorporated herein by reference).

The invention also provides methods for producing a population of antigen presenting cells (APC's), which can express an MG50 T cell epitope complexed with an MHC molecule on their surfaces. APC's

are well known in the art and include dendritic cells, mononuclear phagocytic cells, B lymphocytes, Langerhans cells or human venular cells. In one embodiment of the invention, APC's that contain and express a nucleic acid molecule of the invention are provided. Such a nucleic acid molecule can be introduced into an APC using methods as discussed above. In another embodiment, the APC's are contacted with an MG50 melanoma associated antigen encoded by SEQ ID NO: 1 or a peptide portion thereof, particularly a peptide portion encoded by a nucleotide sequence contained within nucleotides 1 to 5509 or within nucleotides 3555 to 4226 of SEQ ID NO: 1, or are contacted with an MG50 T cell epitope encoded by SEQ ID NO: 1, particularly by a sequence within nucleotides 1 to 5509 or within nucleotides 3555 to 4336 of SEQ ID NO: 1, which can be fused to a signal peptide or a functional portion thereof, if desired. Accordingly, the invention also provides populations of APC's that are produced by a method of the invention and express on their cell surfaces an MG50 T cell epitope complexed with an MHC molecule.

APC's can be contacted, for example, with an MG50 T cell epitope fused to a signal peptide to produce a population of APC's encompassed within the claimed invention. The MG50 T cell epitope is loaded into the cytosol of T cells using osmotic lysis of pinocytic vesicles. T cells exposed to hypertonic medium take-up the fusion peptides due to the formation of pinocytic vesicles in the medium. The pinocytic vesicles break in the cytosol when the cells are placed in hypotonic culture medium, due to the increased internal osmotic pressure. The signal sequence then facilitates translocation of the MG50 T cell epitope from the cytosol into the endoplasmic reticulum, thereby increasing the efficiency with which the epitope is presented at the cell surface complexed with an MHC molecule.

APC's produced by a method of the invention can present an MG50 T cell epitope with a class II molecule or a co-stimulatory B7 molecule to a T cell to activate the T cell. Thus, the invention further provides methods for stimulating T lymphocytes to react specifically against cancer cells expressing an MG50 melanoma associated antigen by contacting the T cells with an APC that presents an MG50 T cell epitope complexed with an MHC molecule on its surface. Although such a stimulation can occur *in vivo*, for example, by administration of the APC's of the invention to an individual, such stimulation of T cells also can be performed *in vitro*. Accordingly, the invention provides an isolated population of T cells, which are specifically reactive with cancer cells that express an MG50 melanoma associated antigen. In addition, it should be recognized that such a population of specifically reactive T cells can be obtained by contacting naive APC's and T cells *in vitro* with MG50 or an MG50 epitope, then isolating the T cells from the APC's. Such *in vitro* methods of producing APC's that express an MG50 T cell epitope complexed with an MHC molecule on its cell surface or of producing T cells specifically reactive with a cell expressing an MG50 melanoma associated antigen are particularly useful because the respected populations of the cells can be expanded such that a large number of the cells can be isolated. Furthermore, the skilled artisan will recognize that the APC's and the T cells can be autologous with respect to each other or can be allogeneic (see, for example, Mitchell, *supra*, 1995).

The invention also provides methods for treating an individual having a cancer containing cancer cells that express an MG50 melanoma associated antigen. It is recognized that the methods of the invention can be curative in some cases. However, a method of the

invention also can be useful where it is palliative and, therefore, increases the quality of life of an individual. In particular, the artisan skilled in cancer therapy will recognize that a method of the invention can be particularly useful in combination with conventional cancer therapeutic modalities, including surgery, radiotherapy and chemotherapy.

An individual having a cancer containing cancer cells that express MG50 can be treated, for example, by administration of APC's that express an MG50 T cell epitope complexed with an MHC molecule on their surfaces. Administration of such APC's can stimulate an active immune response in the subject by presenting MG50 T cell epitopes to the individual's T lymphocytes. In addition, the individual can be treated by administration of T lymphocytes that have been stimulated *in vitro* to react with cancer cells expressing an MG50 melanoma associated antigen, thus providing a means of passive immunization of the individual.

The present invention also provides methods of treating an individual having a cancer containing cancer cells expressing an MG50 melanoma associated antigen by administering an MG50 vaccine to the individual. As used herein, the term "vaccine," when used in reference to the present invention, means a formulation that is suitable for administration to a mammal, particularly a human, and contains an MG50 component selected from 1) an MG50 polypeptide, comprising SEQ ID NO: 2; 2) an MG50 T cell epitope encoded by SEQ ID NO: 1, which can be fused to a signal peptide, if desired; 3) an anti-idiotypic antibody of the invention, which is a mimic of an MG50 epitope; or 4) a nucleic acid molecule encoding an MG50 polypeptide or MG50 T cell epitope.

A vaccine of the invention generally contains a pharmaceutically acceptable carrier, for example, an aqueous solution such as physiologically buffered saline or other solvent or vehicle such as a glycol, glycerol, oil such as olive oil or injectable organic ester. A pharmaceutically acceptable carrier also can include a physiologically acceptable compound that acts, for example, to stabilize the MG50 component of the formulation or to increase the absorption of the MG50 component. Physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients.

If desired, a vaccine of the invention can contain an immunostimulatory agent such as an adjuvant, for example, DETOX (Ribi Immunochem), alum or Freund's complete or incomplete adjuvant. In addition, a vaccine can contain an immunostimulatory agent such as a cytokine, for example, interleukin-2 (IL-2), IL-4, IL-7, IL-12, IL-15, interferon-α (Ifn-α), Ifn-γ, granulocyte-macrophage colony stimulating factor (R&D Systems, Inc.; Minneapolis MN), an accessory molecule such as ICAM-1 or B7, or an agent from plants such as QS-21 (see Mitchell, *supra*, 1995; Jones & Mitchell, *supra*, 1996), although such agents also can be added separately from the vaccine, if desired. Other such agents include vectors that are rendered nonpathogenic, for example, by attenuation, liposomes and cell-sized microspheres (see Jones and Mitchell, *supra*, 1996). The skilled artisan will know how to formulate a vaccine of the invention using methods routine in the art. For example, a vaccine that includes about 10 μg to about 10 mg of MG50 or an MG50 T cell epitope can be administered in conjunction with about 2.5 million units/meter^2 to about 20 million units/meter^2 of interferon-α. Such a combined modality

can be administered about three to five times per week and can be continued for up to about two years.

An MG50 vaccine can be administered for preventive purposes or for therapeutic purposes. The vaccine can be administered for preventive purposes, for example, to minimize the likelihood that a cancer such as melanoma, which expresses MG50, will occur in those individuals that are at high risk for the disease. Such individual include, for example, those suffering from familial dysplastic nevus syndrome or from atypical Spitz nevi, those individuals having a large number of moles or having an irregularly shaped mole, or those individuals living in high incidence regions such as Australia, Hawaii or the southwestern United States. In addition, an MG50 vaccine can be administered for therapeutic purposes to an individual suffering from a cancer that contains cancer cells expressing MG50 and can prevent the further growth or spread of the cancer or induce regression of the cancer. Such cancers can be, for example, melanoma, lung cancer or rhabdomyosarcoma and other cancers expressing MG50 can be identified using methods as disclosed herein.

An MG50 vaccine can be administered in a manner similar to other vaccines, for example, subcutaneously, orally, intradermally, intramuscularly or intravenously. In addition, following a first administration of the vaccine, it can be advantageous to administer one or more booster vaccinations. The need to administer a booster vaccination and the timing of such vaccinations can be determined experimentally by measuring, for example, the presence or proliferation of MG50 reactive Tc cells in the individual.

The MG50 component of a vaccine is administered to the individual in an amount that is sufficient to

stimulate an immune response, particularly a cellular immune response. Such an amount will vary, for example, depending on whether the MG50 component is an MG50 polypeptide or an MG50 T cell epitope or a nucleic acid molecule encoding the MG50 component. In addition, the amount will vary, for example, depending on whether stimulation of the immune response is *in vivo* or *in vitro*; whether the administration is a first administration or a booster administration; whether an immunostimulatory agent such as an adjuvant is administered; and, when administered *in vivo*, on the route of administration. In general, about 10 μg to about 10 mg of an MG50 polypeptide or MG50 T cell epitope is administered per immunization. Methods for determining a sufficient amount of an MG50 component is required to stimulate an immune response are well within the means of the skilled artisan and generally are determined in Phase I and Phase II clinical trials (see, for example, Powell and Newman, Vaccine Design: The subunit and adjuvant approach (Plenum Publ. Corp.; 1994), which is incorporated herein by reference).

Where adminstration is of a nucleic acid molecule encoding MG50 or an MG50 T cell epitope, the nucleic acid molecule can be contained in a vector (see Goeddel, *supra*, 1990). A nucleic acid molecule can be inserted into such a vector using known methods (see, Sambrook, *supra*, 1989). A variety of vectors, including expression vectors, are available and contain, for example, a promoter such as the cytomegalovirus or SV40 promoter, which can direct expression of MG50 or an MG50 T cell epitope in a cell (see, Gacesa and Ramji, Vectors, Essential Data, John Wiley and Sons, NY (1994), which is incorporated herein by reference). Viral vectors based, for example, on a retrovirus, an adenovirus, an adeno-associated virus, a vaccinia virus, or the like,

are particularly useful (see, for example, Anderson et al., U.S. Patent No. 5,399,347, issued March 21, 1995; Lee et al., U.S. Patent No. 5,532,220, issued July 2, 1996; Collins et al., U.S. Patent No. 5,240,846, issued August 31, 1993; and Ram et al., Cancer Res. 53:83-88 (1993); Karlsson et al., EMBO J. 5:2377-2385 (1986); Kleinerman et al., Cancer Res. 55:2831-2836 (1995); Hamada et al., Gynecol. Oncol., 63:219-227 (1996); Nabel et al., Science, 249:1285-1288 (1990); and Berkner, BioTechniques 6:616-629 (1989), each of which is incorporated herein by reference).

In addition to a viral vector, a nucleic acid molecule of the invention can be admininstered using a liposome. Methods of making a liposome containing a nucleic acid molecule are known in the art (see, Nabel et al., Proc. Natl. Acad. Sci. USA, 90:11307-11311 (1993), which is incorporated herein by reference; and Nabel et al., *supra*, 1990). Such liposomes can be made target specific by incorporating, for example, lipid-conjugated antibodies into the structure of the liposome (see, Holmberg et al., J. Liposome Res., 1:393-406 (1990), which is incorporated herein by reference) or by incorporating a ligand or a receptor, that is bound by a corresponding receptor or ligand, respectively, that is expressed on the target cell.

The present invention also provides methods of identifying the presence of an MG50 melanoma associated antigen in an individual. Such a method can be performed, for example, by contacting a biological sample obtained from the individual with an antibody that specifically binds an MG50 epitope, wherein specific binding of the antibody to a component of the sample identifies the presence of the MG50 melanoma associated antigen in the individual. Such a biological sample can be, for example, a tissue or tumor sample, which can be

obtained by a biopsy procedure from an individual suspected of having a cancer in which the cancer cells express an MG50 melanoma associated antigen.

In addition, the invention provides methods of identifying the presence in an immune response against an MG50 melanoma associated antigen in an individual. Such a method can be performed, for example, by contacting a biological sample obtained from the subject with a peptide comprising at least six contiguous amino acids, generally at least 8 contiguous amino acids, encoded by SEQ ID NO: 1 and detecting an immunoeffector function of the sample due to contact with the peptide, thereby identifying the presence of an immune response against an MG50 melanoma associated antigen in the individual. An immunoeffector function can be, for example, the presence of anti-MG50 antibodies in the biological sample or the presence of MG50 reactive T cells in the sample. A biological sample can be, for example, a blood sample or a lymph tissue sample. For example, the peptide can be an MG50 T cell epitope and the ability of epitope to stimulate the proliferation of T cells, which are a component of the biological sample, identifies the presence of MG50 reactive T cells in the sample and, therefore, the presence of an immune response against MG50 in the individual from whom the sample was obtained.

The following examples are provided to illustrate embodiments of the invention.

EXAMPLE I

NUCLEIC ACID MOLECULE ENCODING MG50

This example describes methods for obtaining a nucleic acid molecule encoding the MG50 melanoma associated antigen.

Subtractive hybridization of cDNA obtained from melanoma cell line MSM M-1 ("M1") against an excess of mRNA from a squamous lung carcinoma cell line Lu-1 was used to clone cDNA sequences differentially expressed in the M1 melanoma cells (Hutchins et al., Cancer Research 51:1418-1425 (1991), which is incorporated herein by reference). Twelve candidate differentially expressed clones were obtained, six of which were considered novel based on a lack of sequence homology to sequence in the GenBank database (Hutchins et al., *supra*, 1991). One of these six clones, designated "melanoma gene-50" ("MG50") was selected for further characterization.

Based on northern blot analysis using the MG50 cDNA as a probe, MG50 is encoded by an mRNA of about 8.1 kilobases (kb). MG50 mRNA was detected in melanoma cells, lung carcinoma cells, rhabdomyosarcoma cells, fetal brain, fetal heart and human placenta.

Figure 1 shows a 6848 nucleotide portion of the cDNA encoding MG50 (SEQ ID NO: 1). Nucleotides 5510 to 6848 of SEQ ID NO: 1) were reported previously (Weiler, *supra*, 1993) However, efforts to continue sequencing the cDNA in the 5' direction largely were unsuccessful. As disclosed herein, primers were made based on the 5' end of the portion of the sequence described by Weiler (*supra*, 1993) and used to obtain more 5' sequences, which then were sequenced and used to search the Merck EST database. Overlapping EST sequences were identified and used to extent the sequence of MG50 to nucleotide 4336 of SEQ ID NO: 1 (i.e., nucleotides 43366 to 5509). Additional 5' sequences were determined by anchored PCR, RACE and DNA sequencing to nucleotide 3555 of SEQ ID NO: 1. Recently, the portion SEQ ID NO: 1 shown as nucleotides 1 to 5510 was submitted to GenBank as Accession No. D86983 and, therefore, nucleotides 1 to

3554 were added to produce the sequence shown as SEQ ID NO: 1 (Figures 1A to 1E).

Based on the 8.1 kb mRNA for MG50, it is estimated that approximately 1300 nucleotides remain to be sequenced to obtain the full length MG50 cDNA sequence. At least some of the 1300 nucleotides are expected to be 5' to the sequence shown in SEQ ID NO: 1, since an ATG initiation codon has not yet been identified. The remaining MG50 cDNA sequences can be obtained, for example, using a PCR method such as a RACE method.

A deduced amino acid sequence encoded by nucleotides 1 to 4488 of the MG50 cDNA is shown in Figure 2 (SEQ ID NO: 2). The 1496 amino acid polypeptide shares homology to Drosophila peroxidasin and to products of the human peroxidase gene family. The 1496 amino acid MG50 polypeptide (SEQ ID NO: 2) is shown because the first stop codon encoded by SEQ ID NO: 1 occurs at nucleotides 4489 to 4491. However, if additional amino acids are deduced beyond this stop codon, cryptic coding sequences are revealed. Although additional stop codons are interspersed throughout the cryptic coding region, the sequence is considered to be a cryptic coding region because the peptide RPRPEQEPLP (SEQ ID NO: 4), which is encoded by nucleotides 5410 to 5439 of SEQ ID NO: 1 has the characteristics of an MG50 T cell epitope. Specifically, the peptide of SEQ ID NO: 4 binds more strongly to HLA-B7 than any other epitope tested in a competitive binding assay and stimulates proliferation of $CD8^+$ T cells that were specifically reactive with melanoma cells expressing MG50 (see Example II).

Remarkably, the coding sequence of SEQ ID NO: 4 is downstream of nine stop codons, including the stop codon at nucleotides 4489 to 4491. While the presence of

this T cell epitope (SEQ ID NO: 4) in the cryptic region of SEQ ID NO: 1 may be fortuitous, another possibility is that the cryptic coding region, or a portion of this region, is translated in melanoma cells.

EXAMPLE II

POTENTIAL MG50 T CELL EPITOPES

This example provides peptides that are encoded by SEQ ID NO: 1 and have characteristics of MHC class I restricted T cell epitopes.

The peptide RPRPEQEPLP (SEQ ID NO: 4), which is encoded by nucleotides 5410 to 5439 of SEQ ID NO: 1, in the cryptic region, stimulated proliferation of $CD8^+$ T cells that were specifically reactive with melanoma cells expressing MG50 (see Example II). In these experiments, T2 cells were transduced to express HLA-B7, then incubated with the peptide and $CD8^+$ T cells (Tc cells), which were obtained from a patient having a melanoma that expressed MG50. Proliferation of the T cells indicated that the RPRPEQEPLP (SEQ ID NO: 4) peptide acts as an HLA-B7 restricted Tc cell epitope.

In other experiments, CD8 T cells were generated against the RPRPEQEPLP (SEQ ID NO: 4) peptide *in vitro*, then reacted against RPRPEQEPLP (SEQ ID NO: 4) pulsed Cos-7 cells, which were transduced to express HLA-B7. The Cos-7 cells were lysed, demonstrating that the Tc cells recognized the MG50 T cell epitope in the context of HLA-B7 and were specifically reactive for the peptide.

Potential T cell epitopes encoded within the open reading frame of SEQ ID NO: 1 (i.e., present within SEQ ID NO: 2) were identified by homology to consensus HLA-A1 and HLA-A2 epitope sequences (see Kaat et al.,

supra, 1994; Falk and Rotzschko, *supra*, 1993). As shown in Table 1, a peptide having that characteristics expected of an HLA-A1 epitope (SEQ ID NO: 5) was identified and 12 peptides having characteristics of an HLA-A2 epitope (SEQ ID NOS: 6-17) were identified.

TABLE 1

Amino Acid Sequence	*Amino Acid Position	SEQ ID NO:
CSEQPFPEHTASVQHAD	**	3
RPRPEQEPLP	1801-1810	4
DVTSGNTVY	273-281	5
VLFCAWGTL	34-42	6
CMHLLLEAV	66-74	7
LLLEAVPAV	69-77	8
TLHCDCEIL	210-218	9
VLSVNVPDV	625-633	10
DLDSTVVAL	845-853	11
WLPKILGEV	1051-1059	12
PLLRGLFGV	1133-1141	13
RLGPTLMCL	1244-1252	14
LLSTQFKRL	1252-1260	15
EMQKTITDL	1408-1416	16
DLRTQIKKL	1415-1423	17

* - amino acid position with respect to SEQ ID NO: 2.
** - not encoded by same reading frame as SEQ ID NO: 2.

The ability of the peptides shown in Table 1, or other potential T cell epitope encoded by SEQ ID NO: 1, to act as an MG50 T cell epitope can be determined using a T cell proliferation assay as described above. In addition, Cos-7 cells can be cotransduced with a cDNA

encoding HLA-A1 or HLA-A2, as appropriate, and with a nucleic acid molecule encoding a potential T cell epitope. The cotransduced Cos-7 cells then can be incubated with Tc cells that are specifically reactive with melanoma cells expressing MG50 and MG50 T cell epitopes can be identified by detecting lysis of the Cos-7 cells.

Although the invention has been described with reference to the example provided above, it should be understood that various changes can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California
The University of Southern California

(ii) TITLE OF INVENTION: A Melanoma Associated Antigen, T Cell Epitopes Thereof and Methods of Using Same

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Campbell & Flores LLP
(B) STREET: 4370 La Jolla Village Drive, Suite 700
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: United States
(F) ZIP: 92122

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Campbell, Cathryn A.
(B) REGISTRATION NUMBER: 31,815
(C) REFERENCE/DOCKET NUMBER: FP-UD 3175

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 535-9001
(B) TELEFAX: (619) 535-8949

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6847 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..4489

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AGC CGG CCG TGG TGG CTC CGT GCG TCC GAG CGT CCG TCC GCG CCG TCG          48
Ser Arg Pro Trp Trp Leu Arg Ala Ser Glu Arg Pro Ser Ala Pro Ser
  1               5                  10                  15

GCC ATG GCC AAG CGC TCC AGG GGC CCC GGG CGC CGC TGC CTG TTG GCG          96
Ala Met Ala Lys Arg Ser Arg Gly Pro Gly Arg Arg Cys Leu Leu Ala
            20                  25                  30

CTC GTG CTG TTC TGC GCC TGG GGG ACG CTG GCC GTG GTG GCC CAG AAG         144
Leu Val Leu Phe Cys Ala Trp Gly Thr Leu Ala Val Val Ala Gln Lys
        35                  40                  45

CCG GGC GCA GGG TGT CCG AGC CGC TGC CTG TGC TTC CGC ACC ACC GTG         192
Pro Gly Ala Gly Cys Pro Ser Arg Cys Leu Cys Phe Arg Thr Thr Val
    50                  55                  60

CGC TGC ATG CAT CTG CTG CTG GAG GCC GTG CCC GCC GTG GCG CCG CAG         240
Arg Cys Met His Leu Leu Leu Glu Ala Val Pro Ala Val Ala Pro Gln
65                  70                  75                  80

ACC TCC ATC CTA GAT CTT CGC TTT AAC AGA ATC AGA GAG ATC CAA CCT         288
Thr Ser Ile Leu Asp Leu Arg Phe Asn Arg Ile Arg Glu Ile Gln Pro
                85                  90                  95

GGG GCA TTC AGG CGG CTG AGG AAC TTG AAC ACA TTG CTT CTC AAT AAT         336
Gly Ala Phe Arg Arg Leu Arg Asn Leu Asn Thr Leu Leu Leu Asn Asn
            100                 105                 110

AAT CAG ATC AAG AGG ATA CCT AGT GGA GCA TTT GAA GAC TTG GAA AAT         384
Asn Gln Ile Lys Arg Ile Pro Ser Gly Ala Phe Glu Asp Leu Glu Asn
        115                 120                 125

TTA AAA TAT CTC TAT CTG TAC AAG AAT GAG ATC CAG TCA ATT GAC AGG         432
Leu Lys Tyr Leu Tyr Leu Tyr Lys Asn Glu Ile Gln Ser Ile Asp Arg
    130                 135                 140

CAA GCA TTT AAG GGA CTT GCC TCT CTA GAG CAA CTA TAC CTG CAC TTT         480
Gln Ala Phe Lys Gly Leu Ala Ser Leu Glu Gln Leu Tyr Leu His Phe
145                 150                 155                 160

AAT CAG ATA GAA ACT TTG GAC CCA GAT TCG TTC CAG CAT CTC CCG AAG         528
Asn Gln Ile Glu Thr Leu Asp Pro Asp Ser Phe Gln His Leu Pro Lys
                165                 170                 175

CTC GAG AGG CTA TTT TTG CAT AAC AAC CGG ATT ACA CAT TTA GTT CCA         576
Leu Glu Arg Leu Phe Leu His Asn Asn Arg Ile Thr His Leu Val Pro
            180                 185                 190

GGG ACA TTT AAT CAC TTG GAA TCT ATG AAG AGA TTG CGA CTG GAC TCA         624
Gly Thr Phe Asn His Leu Glu Ser Met Lys Arg Leu Arg Leu Asp Ser
        195                 200                 205

AAC ACA CTT CAC TGC GAC TGT GAA ATC CTG TGG TTG GCG GAT TTG CTG         672
Asn Thr Leu His Cys Asp Cys Glu Ile Leu Trp Leu Ala Asp Leu Leu
    210                 215                 220

AAA ACC TAC GCG GAG TCG GGG AAC GCG CAG GCA GCG GCC ATC TGT GAA         720
Lys Thr Tyr Ala Glu Ser Gly Asn Ala Gln Ala Ala Ala Ile Cys Glu
225                 230                 235                 240
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TAT CCC AGA CGC ATC CAG GGA CGC TCA GTG GCA ACC ATC ACC CCG GAA       768
Tyr Pro Arg Arg Ile Gln Gly Arg Ser Val Ala Thr Ile Thr Pro Glu
                245                 250                 255

GAG CTG AAC TGT GAA AGG CCC CGG ATC ACC TCC GAG CCC CAG GAC GCA       816
Glu Leu Asn Cys Glu Arg Pro Arg Ile Thr Ser Glu Pro Gln Asp Ala
            260                 265                 270

GAT GTG ACC TCG GGG AAC ACC GTG TAC TTC ACC TGC AGA GCC GAA GGC       864
Asp Val Thr Ser Gly Asn Thr Val Tyr Phe Thr Cys Arg Ala Glu Gly
        275                 280                 285

AAC CCC AAG CCT GAG ATC ATC TGG CTG CGA AAC AAT AAT GAG CTG AGC       912
Asn Pro Lys Pro Glu Ile Ile Trp Leu Arg Asn Asn Asn Glu Leu Ser
    290                 295                 300

ATG AAG ACA GAT TCC CGC CTA AAC TTG CTG GAC GAT GGG ACC CTG ATG       960
Met Lys Thr Asp Ser Arg Leu Asn Leu Leu Asp Asp Gly Thr Leu Met
305                 310                 315                 320

ATC CAG AAC ACA CAG GAG ACA GAC CAG GGT ATC TAC CAG TGC ATG GCA      1008
Ile Gln Asn Thr Gln Glu Thr Asp Gln Gly Ile Tyr Gln Cys Met Ala
                325                 330                 335

AAG AAC GTG GCC GGA GAG GTG AAG ACG CAA GAG GTG ACC CTC AGG TAC      1056
Lys Asn Val Ala Gly Glu Val Lys Thr Gln Glu Val Thr Leu Arg Tyr
            340                 345                 350

TTC GGG TCT CCA GCT CGA CCC ACT TTT GTA ATC CAG CCA CAG AAT ACA      1104
Phe Gly Ser Pro Ala Arg Pro Thr Phe Val Ile Gln Pro Gln Asn Thr
        355                 360                 365

GAG GTG CTG GTT GGG GAG AGC GTC ACG CTG GAG TGC AGC GCC ACA GGC      1152
Glu Val Leu Val Gly Glu Ser Val Thr Leu Glu Cys Ser Ala Thr Gly
    370                 375                 380

CAC CCC CCG CCG CGG ATC TCC TGG ACG AGA GGT GAC CGC ACA CCC TTG      1200
His Pro Pro Pro Arg Ile Ser Trp Thr Arg Gly Asp Arg Thr Pro Leu
385                 390                 395                 400

CCA GTT GAC CCG CGG GTG AAC ATC ACG CCT TCT GGC GGG CTT TAC ATA      1248
Pro Val Asp Pro Arg Val Asn Ile Thr Pro Ser Gly Gly Leu Tyr Ile
                405                 410                 415

CAG AAC GTC GTA CAG GGG GAC AGC GGA GAG TAT GCG TGC TCT GCG ACC      1296
Gln Asn Val Val Gln Gly Asp Ser Gly Glu Tyr Ala Cys Ser Ala Thr
            420                 425                 430

AAC AAC ATT GAC AGC GTC CAT GCC ACC GCT TTC ATC ATC GTC CAG GCT      1344
Asn Asn Ile Asp Ser Val His Ala Thr Ala Phe Ile Ile Val Gln Ala
        435                 440                 445

CTT CCT CAG TTC ACT GTG ACG CCT CAG GAC AGA GTC GTT ATT GAG GGC      1392
Leu Pro Gln Phe Thr Val Thr Pro Gln Asp Arg Val Val Ile Glu Gly
    450                 455                 460

CAG ACC GTG GAT TTC CAG TGT GAA GCC AAG GGC AAC CCG CCG CCC GTC      1440
Gln Thr Val Asp Phe Gln Cys Glu Ala Lys Gly Asn Pro Pro Pro Val
465                 470                 475                 480

ATC GCC TGG ACC AAG GGA GGG AGC CAG CTC TCC GTG GAC CGG CGG CAC      1488
Ile Ala Trp Thr Lys Gly Gly Ser Gln Leu Ser Val Asp Arg Arg His
                485                 490                 495
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CTG GTC CTG TCA TCG GGA ACA CTT AGA ATC TCT GGT GTT GCC CTC CAC      1536
Leu Val Leu Ser Ser Gly Thr Leu Arg Ile Ser Gly Val Ala Leu His
            500                 505                 510

GAC CAG GGC CAG TAC GAA TGC CAG GCT GTC AAC ATC ATC GGC TCC CAG      1584
Asp Gln Gly Gln Tyr Glu Cys Gln Ala Val Asn Ile Ile Gly Ser Gln
        515                 520                 525

AAG GTC GTG GCC CAC CTG ACT GTG CAG CCC AGA GTC ACC CCA GTG TTT      1632
Lys Val Val Ala His Leu Thr Val Gln Pro Arg Val Thr Pro Val Phe
    530                 535                 540

GCC AGC ATT CCC AGC GAC ACA ACA GTG GAG GTG GGC GCC AAT GTG CAG      1680
Ala Ser Ile Pro Ser Asp Thr Thr Val Glu Val Gly Ala Asn Val Gln
545                 550                 555                 560

CTC CCG TGC AGC TCC CAG GGC GAG CCC GAG CCA GCC ATC ACC TGG AAC      1728
Leu Pro Cys Ser Ser Gln Gly Glu Pro Glu Pro Ala Ile Thr Trp Asn
                565                 570                 575

AAG GAT GGG GTT CAG GTG ACA GAA AGT GGA AAA TTT CAC ATC AGC CCT      1776
Lys Asp Gly Val Gln Val Thr Glu Ser Gly Lys Phe His Ile Ser Pro
            580                 585                 590

GAA GGA TTC TTG ACC ATC AAT GAC GTT GGC CCT GCA GAC GCA GGT CGC      1824
Glu Gly Phe Leu Thr Ile Asn Asp Val Gly Pro Ala Asp Ala Gly Arg
        595                 600                 605

TAT GAG TGT GTG GCC CGG AAC ACC ATT GGG TCG GCC TCG GTG AGC ATG      1872
Tyr Glu Cys Val Ala Arg Asn Thr Ile Gly Ser Ala Ser Val Ser Met
    610                 615                 620

GTG CTC AGT GTG AAC GTT CCT GAC GTC AGT CGA AAT GGA GAT CCG TTT      1920
Val Leu Ser Val Asn Val Pro Asp Val Ser Arg Asn Gly Asp Pro Phe
625                 630                 635                 640

GTA GCT ACC TCC ATC GTG GAA GCG ATT GCG ACT GTT GAC AGA GCT ATA      1968
Val Ala Thr Ser Ile Val Glu Ala Ile Ala Thr Val Asp Arg Ala Ile
                645                 650                 655

AAC TCA ACC CGA ACA CAT TTG TTT GAC AGC CGT CCT CGT TCT CCA AAT      2016
Asn Ser Thr Arg Thr His Leu Phe Asp Ser Arg Pro Arg Ser Pro Asn
            660                 665                 670

GAT TTG CTG GCC TTG TTC CGG TAT CCG AGG GAT CCT TAC ACA GTT GAA      2064
Asp Leu Leu Ala Leu Phe Arg Tyr Pro Arg Asp Pro Tyr Thr Val Glu
        675                 680                 685

CAG GCA CGG GCG GGA GAA ATC TTT GAA CGG ACA TTG CAG CTC ATT CAG      2112
Gln Ala Arg Ala Gly Glu Ile Phe Glu Arg Thr Leu Gln Leu Ile Gln
    690                 695                 700

GAG CAT GTA CAG CAT GGC TTG ATG GTC GAC CTC AAC GGA ACA AGT TAC      2160
Glu His Val Gln His Gly Leu Met Val Asp Leu Asn Gly Thr Ser Tyr
705                 710                 715                 720

CAC TAC AAC GAC CTG GTG TCT CCA CAG TAC CTG AAC CTC ATC GCA AAC      2208
His Tyr Asn Asp Leu Val Ser Pro Gln Tyr Leu Asn Leu Ile Ala Asn
                725                 730                 735

CTG TCG GGC TGT ACC GCC CAC CGG CGC GTG AAC AAC TGC TCG GAC ATG      2256
Leu Ser Gly Cys Thr Ala His Arg Arg Val Asn Asn Cys Ser Asp Met
            740                 745                 750
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TGC TTC CAC CAG AAG TAC CGG ACG CAC GAC GGC ACC TGT AAC AAC CTG      2304
Cys Phe His Gln Lys Tyr Arg Thr His Asp Gly Thr Cys Asn Asn Leu
        755                 760                 765

CAG CAC CCC ATG TGG GGC GCC TCG CTG ACC GCC TTC GAG CGC CTG CTG      2352
Gln His Pro Met Trp Gly Ala Ser Leu Thr Ala Phe Glu Arg Leu Leu
    770                 775                 780

AAA TCC GTG TAC GAG AAT GGC TTC AAC ACC CCT CGG GGC ATC AAC CCC      2400
Lys Ser Val Tyr Glu Asn Gly Phe Asn Thr Pro Arg Gly Ile Asn Pro
785                 790                 795                 800

CAC CGA CTG TAC AAC GGG CAC GCC CTT CCC ATG CCG CGC CTG GTG TCC      2448
His Arg Leu Tyr Asn Gly His Ala Leu Pro Met Pro Arg Leu Val Ser
                805                 810                 815

ACC ACC CTG ATC GGG ACG GAG ACC GTC ACA CCC GAC GAG CAG TTC ACC      2496
Thr Thr Leu Ile Gly Thr Glu Thr Val Thr Pro Asp Glu Gln Phe Thr
            820                 825                 830

CAC ATG CTG ATG CAG TGG GGC CAG TTC CTG GAC CAC GAC CTC GAC TCC      2544
His Met Leu Met Gln Trp Gly Gln Phe Leu Asp His Asp Leu Asp Ser
        835                 840                 845

ACG GTG GTG GCC CTG AGC CAG GCA CGC TTC TCC GAC GGA CAG CAC TGC      2592
Thr Val Val Ala Leu Ser Gln Ala Arg Phe Ser Asp Gly Gln His Cys
    850                 855                 860

AGC AAC GTG TGC AGC AAC GAC CCC CCC TGC TTC TCT GTC ATG ATC CCC      2640
Ser Asn Val Cys Ser Asn Asp Pro Pro Cys Phe Ser Val Met Ile Pro
865                 870                 875                 880

CCC AAT GAC TCC CGG GCC AGG AGC GGG GCC CGC TGC ATG TTC TTC GTG      2688
Pro Asn Asp Ser Arg Ala Arg Ser Gly Ala Arg Cys Met Phe Phe Val
                885                 890                 895

CGC TCC AGC CCT GTG TGC GGC AGC GGC ATG ACT TCG CTG CTC ATG AAC      2736
Arg Ser Ser Pro Val Cys Gly Ser Gly Met Thr Ser Leu Leu Met Asn
            900                 905                 910

TCC GTG TAC CCG CGG GAG CAG ATC AAC CAG CTC ACC TCC TAC ATC GAC      2784
Ser Val Tyr Pro Arg Glu Gln Ile Asn Gln Leu Thr Ser Tyr Ile Asp
        915                 920                 925

GCA TCC AAC GTG TAC GGG AGC ACG GAG CAT GAG GCC CGC AGC ATC CGC      2832
Ala Ser Asn Val Tyr Gly Ser Thr Glu His Glu Ala Arg Ser Ile Arg
    930                 935                 940

GAC CTG GCC AGC CAC CGC GGC CTG CTG CGG CAG GGC ATC GTG CAG CGG      2880
Asp Leu Ala Ser His Arg Gly Leu Leu Arg Gln Gly Ile Val Gln Arg
945                 950                 955                 960

TCC GGG AAG CCG CTG CTC CCC TTC GCC ACC GGG CCG CCC ACG GAG TGC      2928
Ser Gly Lys Pro Leu Leu Pro Phe Ala Thr Gly Pro Pro Thr Glu Cys
                965                 970                 975

ATG CGG GAC GAG AAC GAG AGC CCC ATC CCC TGC TTC CTG GCC GGG GAC      2976
Met Arg Asp Glu Asn Glu Ser Pro Ile Pro Cys Phe Leu Ala Gly Asp
            980                 985                 990

CAC CGC GCC AAC GAG CAG CTG GGC CTG ACC AGC ATG CAC ACG CTG TGG      3024
His Arg Ala Asn Glu Gln Leu Gly Leu Thr Ser Met His Thr Leu Trp
        995                 1000                1005
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TTC CGC GAG CAC AAC CGC ATT GCC ACG GAG CTG CTC AAG CTG AAC CCG      3072
Phe Arg Glu His Asn Arg Ile Ala Thr Glu Leu Leu Lys Leu Asn Pro
    1010                1015                1020

CAC TGG GAC GGC GAC ACC ATC TAC TAT GAG ACC AGG AAG ATC GTG GGT      3120
His Trp Asp Gly Asp Thr Ile Tyr Tyr Glu Thr Arg Lys Ile Val Gly
1025                1030                1035                1040

GCG GAG ATC CAG CAC ATC ACC TAC CAG CAC TGG CTC CCG AAG ATC CTG      3168
Ala Glu Ile Gln His Ile Thr Tyr Gln His Trp Leu Pro Lys Ile Leu
                1045                1050                1055

GGG GAG GTG GGC ATG AGG ACG CTG GGA GAG TAC CAC GGC TAC GAC CCC      3216
Gly Glu Val Gly Met Arg Thr Leu Gly Glu Tyr His Gly Tyr Asp Pro
            1060                1065                1070

GGC ATC AAT GCT GGC ATC TTC AAC GCC TTC GCC ACC GCG GCC TTC AGG      3264
Gly Ile Asn Ala Gly Ile Phe Asn Ala Phe Ala Thr Ala Ala Phe Arg
        1075                1080                1085

TTT GGC CAC ACG CTT GTC AAC CCA CTG CTT TAC CGG CTG GAC GAG AAC      3312
Phe Gly His Thr Leu Val Asn Pro Leu Leu Tyr Arg Leu Asp Glu Asn
    1090                1095                1100

TTC CAG CCC ATT GCA CAA GAT CAC CTC CCC CTT CAC AAA GCT TTC TTC      3360
Phe Gln Pro Ile Ala Gln Asp His Leu Pro Leu His Lys Ala Phe Phe
1105                1110                1115                1120

TCT CCC TTC CGG ATT GTG AAT GAG GGC GGC ATC GAT CCG CTT CTC AGG      3408
Ser Pro Phe Arg Ile Val Asn Glu Gly Gly Ile Asp Pro Leu Leu Arg
                1125                1130                1135

GGG CTG TTC GGG GTG GCG GGG AAA ATG CGT GTG CCC TCG CAG CTG CTG      3456
Gly Leu Phe Gly Val Ala Gly Lys Met Arg Val Pro Ser Gln Leu Leu
            1140                1145                1150

AAC ACG GAG CTC ACG GAG CGG CTG TTC TCC ATG GCA CAC ACG GTG GCT      3504
Asn Thr Glu Leu Thr Glu Arg Leu Phe Ser Met Ala His Thr Val Ala
        1155                1160                1165

CTG GAC CTG GCG GCC ATC AAC ATC CAG CGG GGC CGG GAC CAC GGG ATC      3552
Leu Asp Leu Ala Ala Ile Asn Ile Gln Arg Gly Arg Asp His Gly Ile
    1170                1175                1180

CCA CCC TAC CAC GAC TAC AGG GTC TAC TGC AAT CTA TCG GCG GCA CAC      3600
Pro Pro Tyr His Asp Tyr Arg Val Tyr Cys Asn Leu Ser Ala Ala His
1185                1190                1195                1200

ACG TTC GAG GAC CTG AAA AAT GAG ATT AAA AAC CCT GAG ATC CGG GAG      3648
Thr Phe Glu Asp Leu Lys Asn Glu Ile Lys Asn Pro Glu Ile Arg Glu
                1205                1210                1215

AAA CTG AAA AGG TTG TAT GGC TCG ACA CTC AAC ATC GAC CTG TTT CCG      3696
Lys Leu Lys Arg Leu Tyr Gly Ser Thr Leu Asn Ile Asp Leu Phe Pro
            1220                1225                1230

GCG CTC GTG GTG GAG GAC CTG GTG CCT GGC AGC CGG CTG GGC CCC ACC      3744
Ala Leu Val Val Glu Asp Leu Val Pro Gly Ser Arg Leu Gly Pro Thr
        1235                1240                1245

CTG ATG TGT CTT CTC AGC ACA CAG TTC AAG CGC CTG CGA GAT GGG GAC      3792
Leu Met Cys Leu Leu Ser Thr Gln Phe Lys Arg Leu Arg Asp Gly Asp
    1250                1255                1260
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AGG TTG TGG TAT GAG AAC CCT GGG GTG TTC TCC CCG GCC CAG CTG ACT      3840
Arg Leu Trp Tyr Glu Asn Pro Gly Val Phe Ser Pro Ala Gln Leu Thr
1265                 1270                 1275                 1280

CAG ATC AAG CAG ACG TCG CTG GCC AGG ATC CTA TGC GAC AAC GCG GAC      3888
Gln Ile Lys Gln Thr Ser Leu Ala Arg Ile Leu Cys Asp Asn Ala Asp
                1285                 1290                 1295

AAC ATC ACC CGG GTG CAG AGC GAC GTG TTC AGG GTG GCG GAG TTC CCT      3936
Asn Ile Thr Arg Val Gln Ser Asp Val Phe Arg Val Ala Glu Phe Pro
            1300                 1305                 1310

CAC GGC TAC GGC AGC TGT GAC GAG ATC CCC AGG GTG GAC CTC CGG GTG      3984
His Gly Tyr Gly Ser Cys Asp Glu Ile Pro Arg Val Asp Leu Arg Val
        1315                 1320                 1325

TGG CAG GAC TGC TGT GAA GAC TGT AGG ACC AGG GGG CAG TTC AAT GCC      4032
Trp Gln Asp Cys Cys Glu Asp Cys Arg Thr Arg Gly Gln Phe Asn Ala
    1330                 1335                 1340

TTT TCC TAT CAT TTC CGA GGC AGA CGG TCT CTT GAG TTC AGC TAC CAG      4080
Phe Ser Tyr His Phe Arg Gly Arg Arg Ser Leu Glu Phe Ser Tyr Gln
1345                 1350                 1355                 1360

GAG GAC AAG CCG ACC AAG AAA ACA AGA CCA CGG AAA ATA CCC AGT GTT      4128
Glu Asp Lys Pro Thr Lys Lys Thr Arg Pro Arg Lys Ile Pro Ser Val
                1365                 1370                 1375

GGG AGA CAG GGG GAA CAT CTC AGC AAC AGC ACC TCA GCC TTC AGC ACA      4176
Gly Arg Gln Gly Glu His Leu Ser Asn Ser Thr Ser Ala Phe Ser Thr
            1380                 1385                 1390

CGC TCA GAT GCA TCT GGG ACA AAT GAC TTC AGA GAG TTT GTT CTG GAA      4224
Arg Ser Asp Ala Ser Gly Thr Asn Asp Phe Arg Glu Phe Val Leu Glu
        1395                 1400                 1405

ATG CAG AAG ACC ATC ACA GAC CTC AGA ACA CAG ATA AAG AAA CTT GAA      4272
Met Gln Lys Thr Ile Thr Asp Leu Arg Thr Gln Ile Lys Lys Leu Glu
    1410                 1415                 1420

TCA CGG CTC AGT ACC ACA GAG TGC GTG GAT GCC GGG GGC GAA TCT CAC      4320
Ser Arg Leu Ser Thr Thr Glu Cys Val Asp Ala Gly Gly Glu Ser His
1425                 1430                 1435                 1440

GCC AAC AAC ACC AAG TGG AAA AAA GAT GCA TGC ACC ATT TGT GAA TGC      4368
Ala Asn Asn Thr Lys Trp Lys Lys Asp Ala Cys Thr Ile Cys Glu Cys
                1445                 1450                 1455

AAA GAC GGG CAG GTC ACC TGC TTC GTG GAA GCT TGC CCC CCT GCC ACC      4416
Lys Asp Gly Gln Val Thr Cys Phe Val Glu Ala Cys Pro Pro Ala Thr
            1460                 1465                 1470

TGT GCT GTC CCC GTG AAC ATC CCA GGG GCC TGC TGT CCA GTC TGC TTA      4464
Cys Ala Val Pro Val Asn Ile Pro Gly Ala Cys Cys Pro Val Cys Leu
        1475                 1480                 1485

CAG AAG AGG GCG GAG GAA AAG CCC T AGGCTCCTGG GAGGCTCCTC             4509
Gln Lys Arg Ala Glu Glu Lys Pro
    1490                 1495

AGAGTTTGTC TGCTGTGCCA TCGTGAGATC GGGTGGCCGA TGGCAGGGAG CTGCGGACTG    4569

CAGACCAGGA AACACCCAGA ACTCGTGACA TTTCATGACA ACGTCCAGCT GGTGCTGTTA    4629
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CAGAAGGCAG TGCAGGAGGC TTCCAACCAG AGCATCTGCG GAGAAGGAGG CACAGCAGGT 4689

GCCTGAAGGG AAGCAGGCAG GAGTCCTAGC TTCACGTTAG ACTTCTCAGG TTTTTATTTA 4749

ATTCTTTTAA AATGAAAAAT TGGTGCTACT ATTAAATTGC ACAGTTGAAT CATTTAGGCG 4809

CCTAAATTGG TTTTGCCTCC CAACACCATT TCTTTTTAAA TAAAGCAGGA TACCTCTATA 4869

TGTCAGCCTT GCCTTGTTCA GATGCCAGGA GCCGGCAGAC CTGTCACCCG CAGGTGGGGT 4929

GAGTCTCGGA GCTGCCAGAG GGGCTCACCG AAATCGGGGT TCCATCACAA GCTATGTTTA 4989

AAAAGAAAAT TGGTGTTTGG CAAACGGAAC AGAACCTTTG ATGAGAGCGT TCACAGGGAC 5049

ACTGTCTGGG GGTGCAGTGC AAGCCCCCGG CCTCTTCCCT GGGAACCTCT GAACTCCTCC 5109

TTCCTCTGGG CTCTCTGTAA CATTTCACCA CACGTCAGCA TCTAATCCCA AGACAAACAT 5169

TCCCGCTGCT CGAAGCAGCT GTATAGCCTG TGACTCTCCG TGTGTCAGCT CCTTCCACAC 5229

CTGATTAGAA CATTCATAAG CCACATTTAG AAACAGATTT GCTTTCAGCT GTCACTTGCA 5289

CACATACTGC CTAGTTGTGA ACCAAATGTG AAAAAACCTC CTTCATCCCA TTGTGTATCT 5349

GATACCTGCC GAGGGCCAAG GGTGTGTGTT GACAACGCCG CTCCCAGCCG GCCCTGGTTG 5409

CGTCCACGTC CTGAACAAGA GCCGCTTCCG GATGGCTCTT CCCAAGGGAG GAGGAGCTCA 5469

AGTGTCGGGA ACTGTCTAAC TTCAGGTTGT GTGAGTGCGT TAAAAAAAAA AAAAAAAAAA 5529

AGAATCCCTA TACCTCATTT GTATTTTTAA AATGCGTGAT GTTTTATGAA ATTGTGTCCA 5589

TTTTTTAGGT ATTAGATATG GCAGAAAAAC CATTTCCACT ATGCAAAGTT CTTTTAGACG 5649

TCAGTGAAAA TCAACTCTCA TACCTCATGG GTCTCTCTTT AATTGACCAA AACCTTCCAT 5709

TTTTCTCTTA AATACAAAGC GATCTGTGTT CTGAGCAACC TTTCCCCGAA CACACAGCTT 5769

CAGTGCAGCA CGCTGACCTG AGTATCCACC AGGTGCCAGG CACAGTTGCT GGGCNNACGG 5829

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GCGTCGAGGC TTCCATGGGG TGGGGGAGGC CAGCTTCCTT CTGACAGGAT GGGCGCATAC 6009

AGTGCCTGGT GTGATTTGTG CACAACCCGT GTTCCAGGTG CACATCCTCC CAAGGAGACA 6069

CCCAGACCCT TCCAGCACGG GCCGGCCAAG TTGCTGCGGC GGAGGCAGCA TTTCAGCTGT 6129

GAGGAAGGTC ATTGGATTCA TGTGTTTTAT CTGTAAAAAT GGTTGTCTTA ACTTCTTAAC 6189

TCATATTGGT AAGTGATTGA TAAAAATTGG TTGGTGTTTT CATGACATGT GGACTTCTNT 6249

TGNATAGAAG TCAAATGTAG TGACAATTTG TGGAAGAGAT TCTTGTCAAA GTGAAATAGG 6309

AAATGTGTAA GTTCGTCTAA AAGCTGATGG TTATGTAAGT TGCTCAGGCA CTCAGATGAC 6369

AGCAGATTCT GGGTTCTGGG AGTGTTCTGT GCCTCTTACA TGCCCTGGAG GCCTCATGGT 6429

CTCAGTGCTG AGGCGGCACA CCTGTAGCAC ACCTGCGTAA TGTGCGGTCT GGGCCAGTCA 6489

CAAGGAATTG TGTTGTCTAA NCCAAAGGGG GAAGCTGACT GTGTATTACC AAAAAAAATT 6549

CTGTAATNCA AACCNAAATG TCTGCGGAAT CACCAGTTTG ATACTCTCTG TAATCAGAGC 6609

AGTNGNCTGA GGGCGGNCAG TNCCTGGGTG AACGTGTCTA GCAGCCACTG TGGGGGATCG 6669

CTGTAACAGG AGTGGAATGT ACATATTTAT TTACTTTTCT AACTGCTCCA ACAGCCAAAT 6729

GCCTTTTTTA TGACCATTGT ATTCAGTTCA TTACCAAAGA AATGTTTGCA CTTTGTAATG 6789

ATGCCTTTCA GTTCAAATAA ATGGGTCACA TTTTCAAATG GAAAAAAAAA AAAAAAAA 6847

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1496 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Arg Pro Trp Trp Leu Arg Ala Ser Glu Arg Pro Ser Ala Pro Ser
1 5 10 15

Ala Met Ala Lys Arg Ser Arg Gly Pro Gly Arg Arg Cys Leu Leu Ala
20 25 30

Leu Val Leu Phe Cys Ala Trp Gly Thr Leu Ala Val Val Ala Gln Lys
35 40 45

Pro Gly Ala Gly Cys Pro Ser Arg Cys Leu Cys Phe Arg Thr Thr Val
50 55 60

Arg Cys Met His Leu Leu Leu Glu Ala Val Pro Ala Val Ala Pro Gln
65 70 75 80

Thr Ser Ile Leu Asp Leu Arg Phe Asn Arg Ile Arg Glu Ile Gln Pro
85 90 95

Gly Ala Phe Arg Arg Leu Arg Asn Leu Asn Thr Leu Leu Leu Asn Asn
100 105 110

Asn Gln Ile Lys Arg Ile Pro Ser Gly Ala Phe Glu Asp Leu Glu Asn
115 120 125

Leu Lys Tyr Leu Tyr Leu Tyr Lys Asn Glu Ile Gln Ser Ile Asp Arg
130 135 140

Gln Ala Phe Lys Gly Leu Ala Ser Leu Glu Gln Leu Tyr Leu His Phe
145 150 155 160

Asn Gln Ile Glu Thr Leu Asp Pro Asp Ser Phe Gln His Leu Pro Lys
165 170 175

Leu Glu Arg Leu Phe Leu His Asn Asn Arg Ile Thr His Leu Val Pro
180 185 190

Gly Thr Phe Asn His Leu Glu Ser Met Lys Arg Leu Arg Leu Asp Ser
195 200 205

Asn Thr Leu His Cys Asp Cys Glu Ile Leu Trp Leu Ala Asp Leu Leu
210 215 220

Lys Thr Tyr Ala Glu Ser Gly Asn Ala Gln Ala Ala Ala Ile Cys Glu
225 230 235 240

Tyr Pro Arg Arg Ile Gln Gly Arg Ser Val Ala Thr Ile Thr Pro Glu
245 250 255

Glu Leu Asn Cys Glu Arg Pro Arg Ile Thr Ser Glu Pro Gln Asp Ala
260 265 270

Asp Val Thr Ser Gly Asn Thr Val Tyr Phe Thr Cys Arg Ala Glu Gly
275 280 285

Asn Pro Lys Pro Glu Ile Ile Trp Leu Arg Asn Asn Asn Glu Leu Ser
290 295 300

Met Lys Thr Asp Ser Arg Leu Asn Leu Leu Asp Asp Gly Thr Leu Met
305 310 315 320

Ile Gln Asn Thr Gln Glu Thr Asp Gln Gly Ile Tyr Gln Cys Met Ala
325 330 335

Lys Asn Val Ala Gly Glu Val Lys Thr Gln Glu Val Thr Leu Arg Tyr
340 345 350

Phe Gly Ser Pro Ala Arg Pro Thr Phe Val Ile Gln Pro Gln Asn Thr
355 360 365

Glu Val Leu Val Gly Glu Ser Val Thr Leu Glu Cys Ser Ala Thr Gly
370 375 380

His Pro Pro Pro Arg Ile Ser Trp Thr Arg Gly Asp Arg Thr Pro Leu
385 390 395 400

Pro Val Asp Pro Arg Val Asn Ile Thr Pro Ser Gly Gly Leu Tyr Ile
405 410 415

Gln Asn Val Val Gln Gly Asp Ser Gly Glu Tyr Ala Cys Ser Ala Thr
420 425 430

Asn Asn Ile Asp Ser Val His Ala Thr Ala Phe Ile Ile Val Gln Ala
435 440 445

Leu Pro Gln Phe Thr Val Thr Pro Gln Asp Arg Val Val Ile Glu Gly
450 455 460

Gln Thr Val Asp Phe Gln Cys Glu Ala Lys Gly Asn Pro Pro Pro Val
465 470 475 480

Ile Ala Trp Thr Lys Gly Gly Ser Gln Leu Ser Val Asp Arg Arg His
485 490 495

Leu Val Leu Ser Ser Gly Thr Leu Arg Ile Ser Gly Val Ala Leu His
500 505 510

Asp Gln Gly Gln Tyr Glu Cys Gln Ala Val Asn Ile Ile Gly Ser Gln
515 520 525

Lys Val Val Ala His Leu Thr Val Gln Pro Arg Val Thr Pro Val Phe
530 535 540

Ala Ser Ile Pro Ser Asp Thr Thr Val Glu Val Gly Ala Asn Val Gln
545 550 555 560

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Leu Pro Cys Ser Ser Gln Gly Glu Pro Glu Pro Ala Ile Thr Trp Asn
                565                 570                 575

Lys Asp Gly Val Gln Val Thr Glu Ser Gly Lys Phe His Ile Ser Pro
            580                 585                 590

Glu Gly Phe Leu Thr Ile Asn Asp Val Gly Pro Ala Asp Ala Gly Arg
        595                 600                 605

Tyr Glu Cys Val Ala Arg Asn Thr Ile Gly Ser Ala Ser Val Ser Met
    610                 615                 620

Val Leu Ser Val Asn Val Pro Asp Val Ser Arg Asn Gly Asp Pro Phe
625                 630                 635                 640

Val Ala Thr Ser Ile Val Glu Ala Ile Ala Thr Val Asp Arg Ala Ile
                645                 650                 655

Asn Ser Thr Arg Thr His Leu Phe Asp Ser Arg Pro Arg Ser Pro Asn
            660                 665                 670

Asp Leu Leu Ala Leu Phe Arg Tyr Pro Arg Asp Pro Tyr Thr Val Glu
        675                 680                 685

Gln Ala Arg Ala Gly Glu Ile Phe Glu Arg Thr Leu Gln Leu Ile Gln
    690                 695                 700

Glu His Val Gln His Gly Leu Met Val Asp Leu Asn Gly Thr Ser Tyr
705                 710                 715                 720

His Tyr Asn Asp Leu Val Ser Pro Gln Tyr Leu Asn Leu Ile Ala Asn
                725                 730                 735

Leu Ser Gly Cys Thr Ala His Arg Arg Val Asn Asn Cys Ser Asp Met
            740                 745                 750

Cys Phe His Gln Lys Tyr Arg Thr His Asp Gly Thr Cys Asn Asn Leu
        755                 760                 765

Gln His Pro Met Trp Gly Ala Ser Leu Thr Ala Phe Glu Arg Leu Leu
    770                 775                 780

Lys Ser Val Tyr Glu Asn Gly Phe Asn Thr Pro Arg Gly Ile Asn Pro
785                 790                 795                 800

His Arg Leu Tyr Asn Gly His Ala Leu Pro Met Pro Arg Leu Val Ser
                805                 810                 815

Thr Thr Leu Ile Gly Thr Glu Thr Val Thr Pro Asp Glu Gln Phe Thr
            820                 825                 830

His Met Leu Met Gln Trp Gly Gln Phe Leu Asp His Asp Leu Asp Ser
        835                 840                 845

Thr Val Val Ala Leu Ser Gln Ala Arg Phe Ser Asp Gly Gln His Cys
    850                 855                 860

Ser Asn Val Cys Ser Asn Asp Pro Pro Cys Phe Ser Val Met Ile Pro
865                 870                 875                 880

Pro Asn Asp Ser Arg Ala Arg Ser Gly Ala Arg Cys Met Phe Phe Val
                885                 890                 895
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Arg Ser Ser Pro Val Cys Gly Ser Gly Met Thr Ser Leu Leu Met Asn
            900                 905                 910

Ser Val Tyr Pro Arg Glu Gln Ile Asn Gln Leu Thr Ser Tyr Ile Asp
        915                 920                 925

Ala Ser Asn Val Tyr Gly Ser Thr Glu His Glu Ala Arg Ser Ile Arg
    930                 935                 940

Asp Leu Ala Ser His Arg Gly Leu Leu Arg Gln Gly Ile Val Gln Arg
945                 950                 955                 960

Ser Gly Lys Pro Leu Leu Pro Phe Ala Thr Gly Pro Pro Thr Glu Cys
                965                 970                 975

Met Arg Asp Glu Asn Glu Ser Pro Ile Pro Cys Phe Leu Ala Gly Asp
            980                 985                 990

His Arg Ala Asn Glu Gln Leu Gly Leu Thr Ser Met His Thr Leu Trp
        995                 1000                1005

Phe Arg Glu His Asn Arg Ile Ala Thr Glu Leu Leu Lys Leu Asn Pro
    1010                1015                1020

His Trp Asp Gly Asp Thr Ile Tyr Tyr Glu Thr Arg Lys Ile Val Gly
1025                1030                1035                1040

Ala Glu Ile Gln His Ile Thr Tyr Gln His Trp Leu Pro Lys Ile Leu
                1045                1050                1055

Gly Glu Val Gly Met Arg Thr Leu Gly Glu Tyr His Gly Tyr Asp Pro
            1060                1065                1070

Gly Ile Asn Ala Gly Ile Phe Asn Ala Phe Ala Thr Ala Ala Phe Arg
        1075                1080                1085

Phe Gly His Thr Leu Val Asn Pro Leu Leu Tyr Arg Leu Asp Glu Asn
    1090                1095                1100

Phe Gln Pro Ile Ala Gln Asp His Leu Pro Leu His Lys Ala Phe Phe
1105                1110                1115                1120

Ser Pro Phe Arg Ile Val Asn Glu Gly Gly Ile Asp Pro Leu Leu Arg
                1125                1130                1135

Gly Leu Phe Gly Val Ala Gly Lys Met Arg Val Pro Ser Gln Leu Leu
            1140                1145                1150

Asn Thr Glu Leu Thr Glu Arg Leu Phe Ser Met Ala His Thr Val Ala
        1155                1160                1165

Leu Asp Leu Ala Ala Ile Asn Ile Gln Arg Gly Arg Asp His Gly Ile
    1170                1175                1180

Pro Pro Tyr His Asp Tyr Arg Val Tyr Cys Asn Leu Ser Ala Ala His
1185                1190                1195                1200

Thr Phe Glu Asp Leu Lys Asn Glu Ile Lys Asn Pro Glu Ile Arg Glu
                1205                1210                1215

Lys Leu Lys Arg Leu Tyr Gly Ser Thr Leu Asn Ile Asp Leu Phe Pro
            1220                1225                1230
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Ala Leu Val Val Glu Asp Leu Val Pro Gly Ser Arg Leu Gly Pro Thr
1235 1240 1245

Leu Met Cys Leu Leu Ser Thr Gln Phe Lys Arg Leu Arg Asp Gly Asp
1250 1255 1260

Arg Leu Trp Tyr Glu Asn Pro Gly Val Phe Ser Pro Ala Gln Leu Thr
1265 1270 1275 1280

Gln Ile Lys Gln Thr Ser Leu Ala Arg Ile Leu Cys Asp Asn Ala Asp
1285 1290 1295

Asn Ile Thr Arg Val Gln Ser Asp Val Phe Arg Val Ala Glu Phe Pro
1300 1305 1310

His Gly Tyr Gly Ser Cys Asp Glu Ile Pro Arg Val Asp Leu Arg Val
1315 1320 1325

Trp Gln Asp Cys Cys Glu Asp Cys Arg Thr Arg Gly Gln Phe Asn Ala
1330 1335 1340

Phe Ser Tyr His Phe Arg Gly Arg Arg Ser Leu Glu Phe Ser Tyr Gln
1345 1350 1355 1360

Glu Asp Lys Pro Thr Lys Lys Thr Arg Pro Arg Lys Ile Pro Ser Val
1365 1370 1375

Gly Arg Gln Gly Glu His Leu Ser Asn Ser Thr Ser Ala Phe Ser Thr
1380 1385 1390

Arg Ser Asp Ala Ser Gly Thr Asn Asp Phe Arg Glu Phe Val Leu Glu
1395 1400 1405

Met Gln Lys Thr Ile Thr Asp Leu Arg Thr Gln Ile Lys Lys Leu Glu
1410 1415 1420

Ser Arg Leu Ser Thr Thr Glu Cys Val Asp Ala Gly Gly Glu Ser His
1425 1430 1435 1440

Ala Asn Asn Thr Lys Trp Lys Lys Asp Ala Cys Thr Ile Cys Glu Cys
1445 1450 1455

Lys Asp Gly Gln Val Thr Cys Phe Val Glu Ala Cys Pro Pro Ala Thr
1460 1465 1470

Cys Ala Val Pro Val Asn Ile Pro Gly Ala Cys Cys Pro Val Cys Leu
1475 1480 1485

Gln Lys Arg Ala Glu Glu Lys Pro
1490 1495

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Ser Glu Gln Pro Phe Pro Glu His Thr Ala Ser Val Gln His Ala
1 5 10 15

Asp

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Pro Arg Pro Glu Gln Glu Pro Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Val Thr Ser Gly Asn Thr Val Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Leu Phe Cys Ala Trp Gly Thr Leu
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys Met His Leu Leu Leu Glu Ala Val
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Leu Leu Glu Ala Val Pro Ala Val
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Leu His Cys Asp Cys Glu Ile Leu
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Leu Ser Val Asn Val Pro Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Asp Leu Asp Ser Thr Val Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Trp Leu Pro Lys Ile Leu Gly Glu Val
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Leu Leu Arg Gly Leu Phe Gly Val
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg Leu Gly Pro Thr Leu Met Cys Leu
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Leu Ser Thr Gln Phe Lys Arg Leu
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu Met Gln Lys Thr Ile Thr Asp Leu
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Leu Arg Thr Gln Ile Lys Lys Leu
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser
20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
1 5 10 15

Met

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Arg Pro Arg Pro Glu Gln Glu Pro Leu Pro
20 25

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Arg Pro Arg Pro Glu Gln Glu Pro Leu Pro
20 25 30

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Ala Arg Pro Arg Pro Glu Gln Glu Pro Leu Pro
20 25

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
1 5 10 15

Met Arg Pro Arg Pro Glu Gln Glu Pro Leu Pro
20 25

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Arg Arg Pro Arg Pro Glu Gln Glu Pro Leu Pro Ala Ala Val Cys
1 5 10 15

Ser Ala

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Ala Arg Pro Arg Pro Glu Gln Glu Pro Leu Pro Ala Ala Ala Ala
1 5 10 15

Ala Gly

We claim:

1. A substantially purified polypeptide portion of a melanoma associated antigen, MG50, comprising the amino acid sequence shown as amino acids 1187 to 1447 of SEQ ID NO: 2.

2. A substantially purified T cell epitope, comprising a contiguous amino acid sequence of SEQ ID NO: 2.

3. The T cell epitope of claim 2, which is a cytotoxic T cell epitope comprising 8 to 11 contiguous amino acids of SEQ ID NO: 2.

4. The T cell epitope of claim 2, comprising the amino acid sequence RPRPEQEPLP (SEQ ID NO: 4).

5. The T cell epitope of claim 2, comprising the amino acid sequence DVTSGNTVY (SEQ ID NO: 5).

6. The T cell epitope of claim 2, comprising an amino acid sequence selected from the group consisting of VLFCAWGTL (SEQ ID NO: 6), CMHLLLEAV (SEQ ID NO: 7), LLLEAVPAV (SEQ ID NO: 8), TLHCDCEIL (SEQ ID NO: 9), VLSVNVPDV (SEQ ID NO: 10), DLDSTVVAL (SEQ ID NO: 11), WLPKILGEV (SEQ ID NO: 12), PLLRGLFGV (SEQ ID NO: 13), RLGPTLMCL (SEQ ID NO: 14), LLSTQFKRL (SEQ ID NO: 15), EMQKTITDL (SEQ ID NO: 16) and DLRTQIKKL (SEQ ID NO: 17).

7. The T cell epitope of claim 2, which encodes a helper T cell epitope comprising 12 to 25 contiguous amino acids of SEQ ID NO: 2.

8. A substantially purified T cell epitope encoded by a nucleotide sequence contained within nucleotides 1 to 5509 of SEQ ID NO: 1.

9. A T cell epitope, comprising a cytotoxic T cell epitope having an amino acid sequence encoded by SEQ ID NO: 1, fused to a signal peptide or a functional portion thereof.

10. The cytotoxic T cell epitope of claim 9, wherein said signal peptide has an amino acid sequence selected from the group consisting of MRYMILGLLALAAVCSA (SEQ ID NO: 18), MTNKCLLQIALLLCFSTTALS (SEQ ID NO: 19), and RYMILGLLALAAVCSAM (SEQ ID NO: 20).

11. The cytotoxic T cell epitope of claim 9, comprising the amino acid sequence RPRPEQEPLP (SEQ ID NO: 4).

12. The cytotoxic T cell epitope of claim 9, which is selected from the group consisting of:

MRYMILGLLALAAVCSARPRPEQEPLP (SEQ ID NO: 21), MTNKCLLQIALLLCFSTTALSRPRPEQEPLP (SEQ ID NO: 22), MRYMILGLLALAAVCSAARPRPEQEPLP (SEQ ID NO: 23), and RYMILGLLALAAVCSAMRPRPEQEPLP (SEQ ID NO: 24).

13. The cytotoxic T cell epitope of claim 9, which is selected from the group consisting of:

MRRPRPEQEPLPAAVCSA (SEQ ID NO: 25), and MARPRPEQEPLPAAAAAG (SEQ ID NO: 26).

14. A chimeric polypeptide, comprising:

a) an MG50 polypeptide encoded by SEQ ID NO: 1 or an MG50 T cell epitope encoded by SEQ ID NO: 1; and

b) a second polypeptide, which is not MG50 or an MG50 T cell epitope.

15. An antibody or an antigen binding fragment thereof that specifically binds an antigen selected from the group consisting of:

a) an MG50 melanoma associated antigen comprising amino acids 1187 to 1447 of SEQ ID NO: 2;

b) a peptide portion of MG50, which is encoded by a nucleotide sequence contained within nucleotides 1 to 5509 of SEQ ID NO: 1;

c) a cytotoxic MG50 T cell epitope encoded by a nucleotide sequence of SEQ ID NO: 1;

d) an MG50 T cell epitope, comprising an amino acid sequence encoded by SEQ ID NO: 1, fused to a signal peptide or a functional portion thereof; and

e) a chimeric polypeptide, comprising an MG50 polypeptide encoded by SEQ ID NO: 1 or an MG50 T cell epitope encoded by SEQ ID NO: 1.

16. The antibody of claim 15, wherein said peptide portion of MG50 is an MG50 T cell epitope.

17. The antibody of claim 15, which is a monoclonal antibody.

18. A cell expressing the antibody of claim 17.

19. An anti-idiotypic antibody, which specifically binds to the antibody of claim 17.

20. A substantially purified nucleic acid molecule, comprising nucleotides 3555 to 4336 of SEQ ID NO: 1.

21. The nucleic acid molecule of claim 20, comprising nucleotides 1 to 6448 of SEQ ID NO: 1.

22. The nucleic acid molecule of claim 20, comprising nucleotides 3555 to 6448 of SEQ ID NO: 1.

23. A substantially purified nucleic acid molecule encoding a polypeptide comprising amino acids 1187 to 1447 of SEQ ID NO: 2.

24. A nucleic acid molecule encoding a T cell epitope of SEQ ID NO: 2.

25. The nucleic acid molecule of claim 24, which encodes a cytotoxic T cell epitope comprising 8 to 11 contiguous amino acids encoded by SEQ ID NO: 1.

26. The nucleic acid molecule of claim 25, comprising the amino acid sequence RPRPEQEPLP (SEQ ID NO: 4).

27. The nucleic acid molecule of claim 25, comprising the amino acid sequence DVTSGNTVY (SEQ ID NO: 5).

28. The nucleic acid molecule of claim 25, comprising an amino acid sequence selected from the group consisting of VLFCAWGTL (SEQ ID NO: 6), CMHLLLEAV (SEQ ID NO: 7), LLLEAVPAV (SEQ ID NO: 8), TLHCDCEIL (SEQ ID NO: 9), VLSVNVPDV (SEQ ID NO: 10), DLDSTVVAL (SEQ ID NO: 11), WLPKILGEV (SEQ ID NO: 12), PLLRGLFGV (SEQ ID NO: 13), RLGPTLMCL (SEQ ID NO: 14), LLSTQFKRL (SEQ ID NO: 15), EMQKTITDL (SEQ ID NO: 16) and DLRTQIKKL (SEQ ID NO: 17).

29. The nucleic acid molecule of claim 24, which encodes a helper T cell epitope comprising 12 to 25 contiguous amino acids encoded by SEQ ID NO: 1.

30. A nucleic acid molecule, comprising a nucleotide sequence encoding a molecule selected from the group consisting of an MG50 T cell epitope fused to a signal peptide; and a chimeric polypeptide, comprising an MG50 polypeptide encoded by SEQ ID NO: 1 or an MG50 T cell epitope encoded by SEQ ID NO: 1.

31. A vector, comprising a nucleic acid molecule encoding a molecule selected from the group consisting of

a) an MG50 polypeptide, comprising amino acids 1187 to 1447 of SEQ ID NO: 2;

b) an MG50 T cell epitope, comprising a contiguous amino acid sequence encoded by a nucleotide sequence contained within nucleotides 1 to 5509 of SEQ ID NO: 1;

c) a cytotoxic MG50 T cell epitope encoded by a nucleotide sequence of SEQ ID NO: 1;

d) an MG50 T cell epitope, comprising a contiguous amino acid sequence encoded by SEQ ID NO: 1, fused to a signal peptide; and

e) a chimeric polypeptide, comprising an MG50 polypeptide encoded by SEQ ID NO: 1 or an MG50 T cell epitope encoded by SEQ ID NO: 1.

32. The vector of claim 31, which is an expression vector.

33. A cell containing the vector of claim 31.

34. The cell of claim 33, which is an antigen presenting cell.

35. The antigen presenting cell of claim 34, which is selected from the group consisting of a dendritic cell, a mononuclear phagocytic cell, a B lymphocyte, a Langerhans cell and a human venular endothelial cell.

36. The cell of claim 33, which expresses the encoded molecule on its surface.

37. A method of identifying the presence of an MG50 melanoma associated antigen in an individual, comprising the steps of:

a) contacting a biological sample obtained from the subject with a ligand that specifically binds MG50; and

b) detecting specific binding of the ligand and the peptide,

wherein the specific binding identifies the presence of the MG50 melanoma associated antigen.

38. The method of claim 37, wherein the ligand is an antibody.

39. A method of identifying the presence in an immune response against an MG50 melanoma associated antigen in an individual, comprising the steps of:

a) contacting a biological sample obtained from the subject with a peptide comprising at least eight contiguous amino acids encoded by SEQ ID NO: 1; and

b) detecting an immunoeffector function of the sample due to contact with the peptide,

wherein the immunoeffector function identifies the presence of an immune response against an MG50 melanoma associated antigen in the individual.

40. The method of claim 39, wherein said immunoeffector function is T cell proliferation.

41. The method of claim 39, wherein said immunoeffector function is specific binding by an antibody.

42. A method for producing a population of antigen presenting cells that express an MG50 T cell epitope complexed with an MHC molecule on their surfaces, comprising contacting antigen presenting cells with an MG50 melanoma associated antigen, provided said melanoma associated antigen is not CSEQPFPEHTASVQHAD (SEQ ID NO: 3).

43. The method of claim 42, wherein said MG50 melanoma associated antigen comprises SEQ ID NO: 2.

44. The method of claim 42, wherein said MG50 melanoma associated antigen comprises an MG50 T cell epitope encoded by SEQ ID NO: 1.

45. The method of claim 42, wherein said MG50 T cell epitope further comprises a signal peptide or a functional portion thereof.

46. The method of claim 42, wherein said T cell epitope comprises the amino acid sequence RPRPEQEPLP (SEQ ID NO: 4).

47. The method of claim 42, wherein said T cell epitope comprises the amino acid sequence DVTSGNTVY (SEQ ID NO: 5).

48. The method of claim 42, wherein said T cell epitope comprises an amino acid sequence selected from the group consisting of VLFCAWGTL (SEQ ID NO: 6), CMHLLLEAV (SEQ ID NO: 7), LLLEAVPAV (SEQ ID NO: 8), TLHCDCEIL (SEQ ID NO: 9), VLSVNVPDV (SEQ ID NO: 10), DLDSTVVAL (SEQ ID NO: 11), WLPKILGEV (SEQ ID NO: 12), PLLRGLFGV (SEQ ID NO: 13), RLGPTLMCL (SEQ ID NO: 14), LLSTQFKRL (SEQ ID NO: 15), EMQKTITDL (SEQ ID NO: 16) and DLRTQIKKL (SEQ ID NO: 17).

49. A population of antigen presenting cells produced by the method of claim 40.

50. A method of producing a population of T lymphocytes specifically reactive against cancer cells expressing an MG50 melanoma associated antigen, comprising contacting T lymphocytes with the antigen presenting cells of claim 49.

51. The method of claim 50, wherein the antigen presenting cells are autologous with respect to the T lymphocytes.

52. The method of claim 50, wherein the antigen presenting cells are allogeneic with respect to the T lymphocytes.

53. The method of claim 50, wherein said contacting T lymphocytes with the antigen presenting cells is performed *in vitro*.

54. A population of T lymphocytes produced by the method of claim 50.

55. The method of claim 50, wherein said contacting T lymphocytes with the antigen presenting cells is performed *in vivo*.

56. A method for treating an individual having a cancer containing cancer cells expressing an MG50 melanoma associated antigen, comprising administering the T lymphocytes of claim 54 to the individual.

57. A method of treating an individual having a cancer containing cancer cells expressing an MG50 melanoma associated antigen, comprising administering the antigen presenting cells of claim 49 to the individual.

58. A method for treating an individual having a cancer containing cancer cells expressing an MG50 melanoma associated antigen, comprising administering a composition comprising an MG50 melanoma associated antigen to the individual, provided said melanoma associated antigen is not CSEQPFPEHTASVTHAD (SEQ ID NO: 3).

59. The method of claim 58, wherein said composition comprises an MG50 polypeptide encoded by SEQ ID NO: 1.

60. The method of claim 58, wherein said composition comprises an MG50 T cell epitope encoded by SEQ ID NO: 1.

61. The method of claim 58, further comprising administering an immunostimulatory agent to the individual.

62. The method of claim 61, wherein said immunostimulatory agent is an adjuvant.

63. The method of claim 61, wherein said adjuvant is DETOX.

64. The method of claim 61, wherein said immunostimulatory agent is a cytokine.

65. The method of claim 64, wherein said cytokine is selected from the group consisting of interleukin-2 and interferon-α.

66. A method for presenting an MG50 T cell epitope on the surface of an antigen presenting cell, comprising contacting the antigen presenting cell with a nucleic acid molecule encoding a molecule selected from the group consisting of:

a) an MG50 polypeptide, comprising amino acids 1187 to 1447 of SEQ ID NO: 2;

b) an MG50 T cell epitope, comprising a contiguous amino acid sequence of SEQ ID NO: 2;

c) a cytotoxic MG50 T cell epitope encoded by SEQ ID NO: 1;

d) an MG50 T cell epitope, comprising a contiguous amino acid sequence encoded by SEQ ID NO: 1, fused to a signal peptide.

67. A population of antigen presenting cells produced by the method of claim 66.

68. A method for treating an individual having cancer cells expressing an MG50 melanoma associated antigen, comprising administering the antigen presenting cells of claim 65 to the individual.

69. A method for treating an individual having cancer cells expressing an MG50 melanoma associated antigen, comprising administering to the individual a composition comprising a nucleic acid molecule encoding a molecule selected from the group consisting of:

a) an MG50 polypeptide, comprising amino acids 1187 to 1447 SEQ ID NO: 2;

b) an MG50 T cell epitope, comprising a contiguous amino acid sequence of SEQ ID NO: 2;

c) a cytotoxic MG50 T cell epitope encoded by SEQ ID NO: 1; and

d) an MG50 T cell epitope, comprising a contiguous amino acid sequence encoded by SEQ ID NO: 1, fused to a signal peptide.

1 AGCCGGCCGT GGTGGCTCCG TGCGTCCGAG CGTCCGTCCG CGCCGTCGGC CATGGCCAAG

61 CGCTCCAGGG GCCCCGGGCG CCGCTGCCTG TTGGCGCTCG TGCTGTTCTG CGCCTGGGGG

121 ACGCTGGCCG TGGTGGCCCA GAAGCCGGGC GCAGGGTGTC CGAGCCGCTG CCTGTGCTTC

181 CGCACCACCG TGCGCTGCAT GCATCTGCTG CTGGAGGCCG TGCCCGCCGT GGCGCCGCAG

241 ACCTCCATCC TAGATCTTCG CTTTAACAGA ATCAGAGAGA TCCAACCTGG GGCATTCAGG

301 CGGCTGAGGA ACTTGAACAC ATTGCTTCTC AATAATAATC AGATCAAGAG GATACCTAGT

361 GGAGCATTTG AAGACTTGGA AAATTTAAAA TATCTCTATC TGTACAAGAA TGAGATCCAG

421 TCAATTGACA-GGCAAGCATT TAAGGGACTT GCCTCTCTAG AGCAACTATA CCTGCACTTT

481 AATCAGATAG AAACTTTGGA CCCAGATTCG TTCCAGCATC TCCCGAAGCT CGAGAGGCTA

541 TTTTTGCATA ACAACCGGAT TACACATTTA GTTCCAGGGA CATTTAATCA CTTGGAATCT

601 ATGAAGAGAT TGCGACTGGA CTCAAACACA CTTCACTGCG ACTGTGAAAT CCTGTGGTTG

661 GCGGATTTGC TGAAAACCTA CGCGGAGTCG GGGAACGCGC AGGCAGCGGC CATCTGTGAA

721 TATCCCAGAC GCATCCAGGG ACGCTCAGTG GCAACCATCA CCCCGGAAGA GCTGAACTGT

781 GAAAGGCCCC GGATCACCTC CGAGCCCCAG GACGCAGATG TGACCTCGGG GAACACCGTG

841 TACTTCACCT GCAGAGCCGA AGGCAACCCC AAGCCTGAGA TCATCTGGCT GCGAAACAAT

901 AATGAGCTGA GCATGAAGAC AGATTCCCGC CTAAACTTGC TGGACGATGG GACCCTGATG

961 ATCCAGAACA CACAGGAGAC AGACCAGGGT ATCTACCAGT GCATGGCAAA GAACGTGGCC

1021 GGAGAGGTGA AGACGCAAGA GGTGACCCTC AGGTACTTCG GGTCTCCAGC TCGACCCACT

1081 TTTGTAATCC AGCCACAGAA TACAGAGGTG CTGGTTGGGG AGAGCGTCAC GCTGGAGTGC

1141 AGCGCCACAG GCCACCCCCC GCCGCGGATC TCCTGGACGA GAGGTGACCG CACACCCTTG

1201 CCAGTTGACC CGCGGGTGAA CATCACGCCT TCTGGCGGGC TTTACATACA GAACGTCGTA

1261 CAGGGGGACA GCGGAGAGTA TGCGTGCTCT GCGACCAACA ACATTGACAG CGTCCATGCC

1321 ACCGCTTTCA TCATCGTCCA GGCTCTTCCT CAGTTCACTG TGACGCCTCA GGACAGAGTC

1381 GTTATTGAGG GCCAGACCGT GGATTTCCAG TGTGAAGCCA AGGGCAACCC GCCGCCCGTC

1441 ATCGCCTGGA CCAAGGGAGG GAGCCAGCTC TCCGTGGACC GGCGGCACCT GGTCCTGTCA

1501 TCGGGAACAC TTAGAATCTC TGGTGTTGCC CTCCACGACC AGGGCCAGTA CGAATGCCAG

FIG. 1A

1561 GCTGTCAACA TCATCGGCTC CCAGAAGGTC GTGGCCCACC TGACTGTGCA GCCCAGAGTC

1621 ACCCCAGTGT TTGCCAGCAT TCCCAGCGAC ACAACAGTGG AGGTGGGCGC CAATGTGCAG

1681 CTCCCGTGCA GCTCCCAGGG CGAGCCCGAG CCAGCCATCA CCTGGAACAA GGATGGGGTT

1741 CAGGTGACAG AAAGTGGAAA ATTTCACATC AGCCCTGAAG GATTCTTGAC CATCAATGAC

1801 GTTGGCCCTG CAGACGCAGG TCGCTATGAG TGTGTGGCCC GGAACACCAT TGGGTCGGCC

1861 TCGGTGAGCA TGGTGCTCAG TGTGAACGTT CCTGACGTCA GTCGAAATGG AGATCCGTTT

1921 GTAGCTACCT CCATCGTGGA AGCGATTGCG ACTGTTGACA GAGCTATAAA CTCAACCCGA

1981 ACACATTTGT TTGACAGCCG TCCTCGTTCT CCAAATGATT TGCTGGCCTT GTTCCGGTAT

2041 CCGAGGGATC CTTACACAGT TGAACAGGCA CGGGCGGGAG AAATCTTTGA ACGGACATTG

2101 CAGCTCATTC AGGAGCATGT ACAGCATGGC TTGATGGTCG ACCTCAACGG AACAAGTTAC

2161 CACTACAACG ACCTGGTGTC TCCACAGTAC CTGAACCTCA TCGCAAACCT GTCGGGCTGT

2221 ACCGCCCACC GGCGCGTGAA CAACTGCTCG GACATGTGCT TCCACCAGAA GTACCGGACG

2281 CACGACGGCA CCTGTAACAA CCTGCAGCAC CCCATGTGGG GCGCCTCGCT GACCGCCTTC

2341 GAGCGCCTGC TGAAATCCGT GTACGAGAAT GGCTTCAACA CCCCTCGGGG CATCAACCCC

2401 CACCGACTGT ACAACGGGCA CGCCCTTCCC ATGCCGCGCC TGGTGTCCAC CACCCTGATC

2461 GGGACGGAGA CCGTCACACC CGACGAGCAG TTCACCCACA TGCTGATGCA GTGGGGCCAG

2521 TTCCTGGACC ACGACCTCGA CTCCACGGTG GTGGCCCTGA GCCAGGCACG CTTCTCCGAC

2581 GGACAGCACT GCAGCAACGT GTGCAGCAAC GACCCCCCCT GCTTCTCTGT CATGATCCCC

2641 CCCAATGACT CCCGGGCCAG GAGCGGGGCC CGCTGCATGT TCTTCGTGCG CTCCAGCCCT

2701 GTGTGCGGCA GCGGCATGAC TTCGCTGCTC ATGAACTCCG TGTACCCGCG GGAGCAGATC

2761 AACCAGCTCA CCTCCTACAT CGACGCATCC AACGTGTACG GGAGCACGGA GCATGAGGCC

2821 CGCAGCATCC GCGACCTGGC CAGCCACCGC GGCCTGCTGC GGCAGGGCAT CGTGCAGCGG

2881 TCCGGGAAGC CGCTGCTCCC CTTCGCCACC GGGCCGCCCA CGGAGTGCAT GCGGGACGAG

2941 AACGAGAGCC CCATCCCCTG CTTCCTGGCC GGGGACCACC GCGCCAACGA GCAGCTGGGC

3001 CTGACCAGCA TGCACACGCT GTGGTTCCGC GAGCACAACC GCATTGCCAC GGAGCTGCTC

3061 AAGCTGAACC CGCACTGGGA CGGCGACACC ATCTACTATG AGACCAGGAA GATCGTGGGT

3121 GCGGAGATCC AGCACATCAC CTACCAGCAC TGGCTCCCGA AGATCCTGGG GGAGGTGGGC

FIG. 1B

3181 ATGAGGACGC TGGGAGAGTA CCACGGCTAC GACCCCGGCA TCAATGCTGG CATCTTCAAC

3241 GCCTTCGCCA CCGCGGCCTT CAGGTTTGGC CACACGCTTG TCAACCCACT GCTTTACCGG

3301 CTGGACGAGA ACTTCCAGCC CATTGCACAA GATCACCTCC CCCTTCACAA AGCTTTCTTC

3361 TCTCCCTTCC GGATTGTGAA TGAGGGCGGC ATCGATCCGC TTCTCAGGGG GCTGTTCGGG

3421 GTGGCGGGGA AAATGCGTGT GCCCTCGCAG CTGCTGAACA CGGAGCTCAC GGAGCGGCTG

3481 TTCTCCATGG CACACACGGT GGCTCTGGAC CTGGCGGCCA TCAACATCCA GCGGGGCCGG

3541 GACCACGGGA TCCCACCCTA CCACGACTAC AGGGTCTACT GCAATCTATC GGCGGCACAC

3601 ACGTTCGAGG ACCTGAAAAA TGAGATTAAA AACCCTGAGA TCCGGGAGAA ACTGAAAAGG

3661 TTGTATGGCT CGACACTCAA CATCGACCTG TTTCCGGCGC TCGTGGTGGA GGACCTGGTG

3721 CCTGGCAGCC GGCTGGGCCC CACCCTGATG TGTCTTCTCA GCACACAGTT CAAGCGCCTG

3781 CGAGATGGGG ACAGGTTGTG GTATGAGAAC CCTGGGGTGT TCTCCCCGGC CCAGCTGACT

3841 CAGATCAAGC AGACGTCGCT GGCCAGGATC CTATGCGACA ACGCGGACAA CATCACCCGG

3901 GTGCAGAGCG ACGTGTTCAG GGTGGCGGAG TTCCCTCACG GCTACGGCAG CTGTGACGAG

3961 ATCCCCAGGG TGGACCTCCG GGTGTGGCAG GACTGCTGTG AAGACTGTAG GACCAGGGGG

4021 CAGTTCAATG CCTTTTCCTA TCATTTCCGA GGCAGACGGT CTCTTGAGTT CAGCTACCAG

4081 GAGGACAAGC CGACCAAGAA AACAAGACCA CGGAAAATAC CCAGTGTTGG GAGACAGGGG

4141 GAACATCTCA GCAACAGCAC CTCAGCCTTC AGCACACGCT CAGATGCATC TGGGACAAAT

4201 GACTTCAGAG AGTTTGTTCT GGAAATGCAG AAGACCATCA CAGACCTCAG AACACAGATA

4261 AAGAAACTTG AATCACGGCT CAGTACCACA GAGTGCGTGG ATGCCGGGGG CGAATCTCAC

4321 GCCAACAACA CCAAGTGGAA AAAAGATGCA TGCACCATTT GTGAATGCAA AGACGGGCAG

4381 GTCACCTGCT TCGTGGAAGC TTGCCCCCCT GCCACCTGTG CTGTCCCCGT GAACATCCCA

4441 GGGGCCTGCT GTCCAGTCTG CTTACAGAAG AGGGCGGAGG AAAAGCCCTA GGCTCCTGGG

4501 AGGCTCCTCA GAGTTTGTCT GCTGTGCCAT CGTGAGATCG GGTGGCCGAT GGCAGGGAGC

4561 TGCGGACTCG AGACCAGGAA ACACCCAGAA CTCGTGACAT TTCATGACAA CGTCCAGCTG

4621 GTGCTGTTAC AGAAGGCAGT GCAGGAGGCT TCCAACCAGA GCATCTGCGG AGAAGGAGGC

4681 ACAGCAGGTG CCTGAAGGGA AGCAGGCAGG AGTCCTAGCT TCACGTTAGA CTTCTCAGGT

4741 TTTTATTTAA TTCTTTTAAA ATGAAAAATT GGTGCTACTA TTAAATTGCA CAGTTGAATC

FIG. 1C

4801 ATTTAGGCGC CTAAATTGGT TTTGCCTCCC AACACCATTT CTTTTTAAAT AAAGCAGGAT

4861 ACCTCTATAT GTCAGCCTTG CCTTGTTCAG ATGCCAGGAG CCGGCAGACC TGTCACCCGC

4921 AGGTGGGGTG AGTCTCGGAG CTGCCAGAGG GGCTCACCGA AATCGGGGTT CCATCACAAG

4981 CTATGTTTAA AAAGAAAATT GGTGTTTGGC AAACGGAACA GAACCTTTGA TGAGAGCGTT

5041 CACAGGGACA CTGTCTGGGG GTGCAGTGCA AGCCCCCGGC CTCTTCCCTG GGAACCTCTG

5101 AACTCCTCCT TCCTCTGGGC TCTCTGTAAC ATTTCACCAC ACGTCAGCAT CTAATCCCAA

5161 GACAAACATT CCCGCTGCTC GAAGCAGCTG TATAGCCTGT GACTCTCCGT GTGTCAGCTC

5221 CTTCCACACC TGATTAGAAC ATTCATAAGC CACATTTAGA AACAGATTTG CTTTCAGCTG

5281 TCACTTGCAC ACATACTGCC TAGTTGTGAA CCAAATGTGA AAAAACCTCC TTCATCCCAT

5341 TGTGTATCTG ATACCTGCCG AGGGCCAAGG GTGTGTGTTG ACAACGCCGC TCCCAGCCGG

5401 CCCTGGTTGC GTCCACGTCC TGAACAAGAG CCGCTTCCGG ATGGCTCTTC CCAAGGGAGG

5461 AGGAGCTCAA GTGTCGGGAA CTGTCTAACT TCAGGTTGTG TGAGTGCGTT AAAAAAAAAA

5521 AAAAAAAAAA GAATCCCTAT ACCTCATTTG TATTTTTAAA ATGCGTGATG TTTTATGAAA

5581 TTGTGTCCAT TTTTTAGGTA TTAGATATGG CAGAAAAACC ATTTCCACTA TGCAAAGTTC

5641 TTTTAGACGT CAGTGAAAAT CAACTCTCAT ACCTCATGGG TCTCTCTTTA ATTGACCAAA

5701 ACCTTCCATT TTTCTCTTAA ATACAAAGCG ATCTGTGTTC TGAGCAACCT TTCCCCGAAC

5761 ACACAGCTTC AGTGCAGCAC GCTGACCTGA GTATCCACCA GGTGCCAGGC ACAGTTGCTG

5821 GGCNNACGGA GGCACCAAGG TCCGGGCCAC CTGCCCGCAG GCAAGGCCCA GCTGAGGTGG

5881 TGGGAGGGGA GCCCCTGAGG TCAGGGGCCG TTTCGGTTCA GGGTGGCAGG TGTCCAGCAC

5941 TGGGGTATGG CGTCGAGGCT TCCATGGCGT GGGGGAGGCC AGCTTCCTTC TGACAGGATG

6001 GGCGCATACA GTGCCTGGTG TGATTTGTGC ACAACCCGTG TTCCAGGTGC ACATCCTCCC

6061 AAGGAGACAC CCAGACCCTT CCAGCACGGG CCGGCCAAGT TGCTGCGGCG GAGGCAGCAT

6121 TTCAGCTGTG AGGAAGGTCA TTGGATTCAT GTGTTTTATC TGTAAAAATG GTTGTCTTAA

6181 CTTCTTAACT CATATTGGTA AGTGATTGAT AAAAATTGGT TGGTGTTTTC ATGACATGTG

6241 GACTTCTNTT GNATAGAAGT CAAATGTAGT GACAATTTGT GGAAGAGATT CTTGTCAAAG

6301 TGAAATAGGA AATGTGTAAG TTCGTCTAAA AGCTGATGGT TATGTAAGTT GCTCAGGCAC

6361 TCAGATGACA GCAGATTCTG GGTTCTGGGA GTGTTCTGTG CCTCTTACAT GCCCTGGAGG

FIG. 1D

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6421 CCTCATGGTC TCAGTGCTGA GGCGGCACAC CTGTAGCACA CCTGCGTAAT GTGCGGTCTG
6481 GGCCAGTCAC AAGGAATTGT GTTGTCTAAN CCAAAGGGGG AAGCTDACTG TGTATTACCA
6541 AAAAAAATTC TGTAATNCAA ACCNAAATGT CTGCGGAATC ACCAGTTTGA TACTCTCTGT
6601 AATCAGAGCA GTNGNCTGAG GGCGGNCAGT NCCTGGGTGA ACGTGTCTAG CAGCCACTGT
6661 GGGGGATCGC TGTAACAGGA GTGGAATGTA CATATTTATT TACTTTTCTA ACTGCTCCAA
6721 CAGCCAAATG CCTTTTTTAT GACCATTGTA TTCAGTTCAT TACCAAAGAA ATGTTTGCAC
6781 TTTGTAATGA TGCCTTTCAG TTCAAATAAA TGGGTCACAT TTTCAAATGG AAAAAAAAAA
6841 AAAAAAA
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FIG. 1E

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1 SRPWWLRASE RPSAPSAMAK RSRGPGRRCL LALVLFCAWG TLAVVAQKPG AGCPSRCLCF   61
  RTTVRCMHLL LEAVPAVAPQ TSILDLRENR IREIQPGAFR RLRNLNTLLL NNNQIKRIPS  121
  GAFEDLENLK YLYLYKNEIQ SIDRQAFKGL ASLEQLYLHF NQIETLDPDS FQHLPKLERL  181
  FLHNNRITHL VPGTFNHLES MKRLRLDSNT LHCDCEILWL ADLLKTYAES GNAQAAAICE  241
  YPRRIQGRSV ATITPEELNC ERPRITSEPQ DADVTSGNTV YFTCRAEGNP KPEIIWLRNN  301
  NELSMKTDSR LNLLDDGTLM IQNTQETDQG IYQCMAKNVA GEVKTQEVTL RYFGSPARPT  361
  FVIQPQNTEV LVGESVTLEC SATGHPPPRI SWTRGDRTPL PVDPRVNITP SGGLYIQNVV  421
  QGDSGEYACS ATNNIDSVHA TAFIIVQALP QFTVTPQDRV VIEGQTVDFQ CEAKGNPPPV  481
  IAWTKGGSQL SVDRRHLVLS SGTLRISGVA LHDQGQYECQ AVNIIGSQKV VAHLTVQPRV  541
  TPVFASIPSD TTVEVGANVQ LPCSSQGEPE PAITWNKDGV QVTESGKFHI SPEGFLTIND  601
  VGPADAGRYE CVARNTIGSA SVSMVLSVNV PDVSRNGDPF VATSIVEAIA TVDRAINSTR  661
  THLFDSRPRS PNDLLALFRY PRDPYTVEQA RAGEIFERTL QLIQEHVQHG LMVDLNGTSY  721
  HYNDLVSPQY LNLIANLSGC TAHRRVNNCS DMCFHQKYRT HDGTCNNLQH PMWGASLTAF  781
  ERLLKSVYEN GFNTPRGINP HRLYNGHALP MPRLVSTTLI GTETVTPDEQ FTHMLMQWGQ  841
  FLDHDLDSTV VALSQARFSD GQHCSNVCSN DPPCFSVMIP PNDSRARSGA RCMFFVRSSP  901
  VCGSGMTSLL MNSVYPREQI NQLTSYIDAS NVYGSTEHEA RSIRDLASHR GLLRQGIVQR  961
  SGKPLLPFAT GPPTECMRDE NESPIPCFLA GDHRANEQLG LTSMHTLWFR EHNRIATELL 1021
  KLNPHWDGDT IYYETRKIVG AEIQHITYQH WLPKILGEVG MRTLGEYHGY DPGINAGIFN 1081
  AFATAAFRFG HTLVNPLLYR LDENFQPIAQ DHLPLHKAFF SPFRIVNEGG IDPLLRGLFG 1141
  VAGKMRVPSQ LLNTELTERL FSMAHTVALD LAAINIQRGR DHGIPPYHDY RVYCNLSAAH 1201
  TFEDLKNEIK NPEIREKLKR LYGSTLNIDL FPALVVEDLV PGSRLGPTLM CLLSTQFKRL 1261
  RDGDRLWYEN PGVFSPAQLT QIKQTSLARI LCDNADNITR VQSDVFRVAE FPHGYGSCDE 1321
  IPRVDLRVWQ DCCEDCRTRG QFNAFSYHFR GRRSLEFSYQ EDKPTKKTRP RKIPSVGRQG 1381
  EHLSNSTSAF STRSDASGTN DFREFVLEMQ KTITDLRTQI KKLESRLSTT ECVDAGGESH 1441
  ANNTKWKKDA CTICECKDGQ VTCFVEACPP ATCAVPVNIP GACCPVCLQK RAEEKP
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FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11533

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 45/05; C07K 14/00, 14/82; C12N 15/00; C12Q 1/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1, 277; 530/300, 328, 350, 395; 435/7.1, 69.3, 172.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EMBL, GENBANK nucleic acid sequence search of SEQ ID NO: 1; GENESEQ32 amino acid sequence search of SEQ ID NO: 2 and residues 1187-1447 of SEQ ID NO: 2 and SEQ ID NOS: 3-26.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/40907 A1 (GENETICS INSTITUTE, INC.) 19 December 1996, see entire document.	1-14, 20-32, 39-41, 50-56
Y	WO 94/21680 A1 (THE GOVERNMENT OF THE UNITED STATES OF AMERICA) 29 September 1994, see entire document.	1-14, 20-32, 39-41, 50-56

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 AUGUST 1998	13 OCT 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
Thomas Cunningham
Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11533

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-14, 20-32, 39-41, 50-56

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11533

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/93.71, 185.1, 277.1; 530/300, 328, 350, 395; 435/7.1, 69.3, 172.1; 536/23.5

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-14 and 39-41, drawn to peptides/polypeptides and the first method of using said peptides/polypeptides.
Group II, claim(s) 15-19, drawn to antibodies and hybridoma cell lines.
Group III, claim(s) s 20-32, drawn to nucleic acid and nucleic acid vectors.
Group IV, claims 33-36 and 49, drawn to antigen presenting cells.
Group V, claims 37-38, drawn to methods of detecting using ligands such as antibodies.
Group VI, claims 42-48, drawn to a method for producing antigen presenting cells.
Group VII, claims 50-55, drawn to methods of making T cells.
Group VIII, claims 56, drawn to method of treatment using T cells.
Group IX, claims 57, drawn to method of treatment using antigen presenting cells.
Group X, claims 58-65, drawn to method of treatment using MG50 peptides/polypeptides.

Group I of this application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1 and lack a common core structure. In order for more than one species within Group I to be searched, the appropriate additional search fees must be paid. The species are as follows:

Species I(a) (residues 1187-1447 SEQ ID NO:2), species I(b) (SEQ ID NO:2), species I(c)(SEQ ID NO:3), species I(d)(SEQ ID NO:4), species I(e)(SEQ ID NO: 5), species I(f)(SEQ ID NO:6), species I(g)(SEQ ID NO:7), species I(h) through species I(z) corresponding to SEQ ID NOS 8-26, respectively. The peptide which comprises amino acid residues 1187-1447 of SEQ ID NO:2 (species I(a)) is the first mentioned species and will be searched with no additional charge. Claims 1-14 and 39-41 all appear to encompass each species due to the open claim language.

Group II of this application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1 and lack a common core structure. In order for more than one species within Group I to be searched, the appropriate additional search fees must be paid. The species are as follows antibodies which bind to the following species of peptides:

Species II(a) (antibodies binding to residues 1187-1447 SEQ ID NO:2), species I(b) (antibodies binding to SEQ ID NO:2), species II(c)(antibodies binding to SEQ ID NO:3), species II(d)(antibodies binding to SEQ ID NO:4), species II(e)(antibodies binding to SEQ ID NO: 5), species II(f)(antibodies binding to SEQ ID NO:6), species II(g)(antibodies binding to SEQ ID NO:7), species II(h) through species II(z) corresponding to antibodies binding to SEQ ID NOS 8-26, respectively. Species II(a) peptide which comprises amino acid residues 1187-1447 of SEQ ID NO:2 (species II(a)) is the first mentioned species for Group II and will be searched with no additional charge upon payment for Group II. Claims 15-19 all appear to encompass each species due to open claim language.

Group III of this application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1 and lack a common core structure. In order for more than one species within Group I to be searched, the appropriate additional search fees must be paid. The species are as follows nucleic acids encoding the following species of peptides:

Species III(a) (nucleic acids comprising nucleotides 3555-4336, 1-6448, or 3555-6448 of SEQ ID NO:1; species III(b) nucleic acids encoding 1187-1447 SEQ ID NO:2), species III(c) nucleic acids encoding SEQ ID NO:2, species III(d) nucleic acids encoding SEQ ID NO:3, species III(e) nucleic acids encoding SEQ ID NO:4, species III (f)nucleic acids encoding SEQ ID NO: 5, species III(g) nucleic acids encoding SEQ ID NO:), species III(h) nucleic acids encoding SEQ ID NO:7, species III(i) through species III(z) and III(aa) corresponding to nucleic acids encoding SEQ ID NOS 8-26, respectively. Species III(a) is the first mentioned species for Group III and will be searched with no additional charge

upon payment for Group III. Claims 20-32 all appear to encompass each species due to the open claim language.

The claims are deemed to correspond to the species listed above in the following manner:

The following claims are generic: for Group I: 1-14 and 39-41. For Group II: 15-19; for Group III: 20-32.

The inventions listed as Groups I-X do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Each group is directed to products which lack the same or corresponding technical features because they have different structures and functions, e.g. polypeptides, antibodies, nucleic acids, T cells, or antigen presenting cells. The groups directed to methods lack corresponding technical features because they recited different method steps are require the use of products which lack corresponding technical features.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

Each species of peptide/polypeptide lacks the same or corresponding technical features because they have different structures and functions, e.g. correspond to different epitopes.